MCIC '21

The 12th International Multi-Conference on Complexity, Informatics and Cybernetics

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PROCEEDINGS

Volume II

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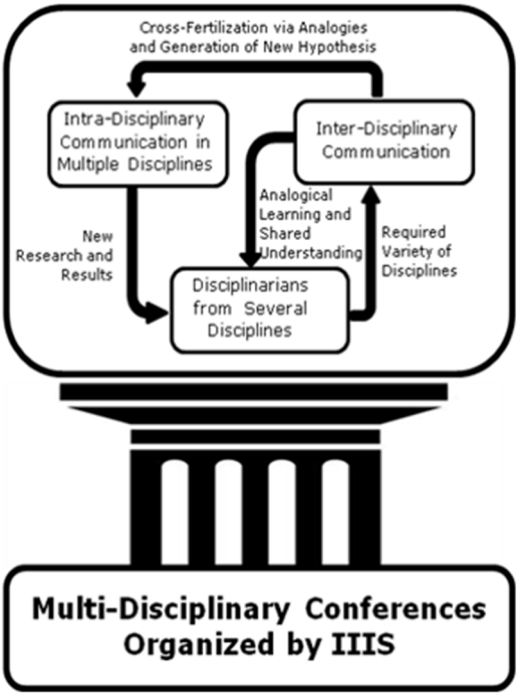
FOREWORD

Our purpose in organizing the 12th International Multi-Conference on Complexity, Informatics and Cybernetics (IMCIC 2021) jointly with the 12th International Conference on Society and Information Technologies (ICSIT 2021) and their collocated events, including the 11th Ibero-American Conference on Complexity, Informatics and Cybernetics (CICIC 2021), is to provide a multi-disciplinary forum for both disciplinary and inter-disciplinary communication.

These collocated events were organized and sponsored by the International Institute of Informatics and Systemics (IIIS, www.iiis.org), member of the International Federation for Systems Research (IFSR, www.ifsr.org). The IIIS is a **multi-disciplinary organization for inter-disciplinary communication and integration**, which includes about 5000 members. Consequently, a main purpose of the IIIS is to foster knowledge integration processes, interdisciplinary communication, and integration of academic activities. Based on: 1) the transdisciplinarity of the systemic approach and its emphasis on *relationships* and *integrating* processes, and 2) the multi-disciplinary support of cybernetics' and informatics' concepts, notions, theories, technologies, and tools, the IIIS has been organizing multi-disciplinary conferences as a platform for fostering inter-disciplinary communication and knowledge integration processes.



Multi-disciplinary conferences are organized by the IIIS as support for both **intra-** and **inter-disciplinary** communication. Processes of intra-disciplinary communication are mainly achieved via traditional paper presentations in corresponding disciplines, while conversational sessions, regarding trans- and inter-disciplinary topics, are among the means used for inter-disciplinary communication. Intra- and inter-disciplinary communications might generate *co-regulative cybernetic loops*, via negative feedback, and *synergic* relationships, via positive feedback loops, in which both kinds of communications could increase their respective effectiveness. Figure (at the right side) shows at least two cybernetic loops if intra- and inter-disciplinary are adequately related. A necessary condition for the effectiveness of Inter-disciplinary communication is an adequate level of **variety** regarding the participating disciplines. *Analogical thinking and learning processes* of disciplinarians depend on it; which in turn are potential sources of the creative tension required for cross-fertilization among disciplines and the generations of new hypothesis. An extended presentation regarding this issue can be found at http://www.iiis.org/MainPurpose/

In general, IIIS is an organization dedicated to contributing to the development of the Systems

Approach, Cybernetics, and Informatics potential, using both: knowledge and experience, thinking and action, for the:

a. identification of synergetic relationships among Systemics, Cybernetics and Informatics, and between them and society;
b. promotion of contacts among the different academic areas, through the transdisciplinarity of the systems approach;
c. identification and implementation of communication channels among the different professions;
d. supply of communication links between the academic and professional worlds, as well as between them and the business world, both public and private, political and cultural;
e. stimulus for the creation of integrative arrangements at different levels of society, as well as at the family and personal levels;
f. promotion of transdisciplinary research, both on theoretical issues and on applications to concrete problems.

Our objective organizing the 12th International Multi-Conference on Complexity, Informatics and Cybernetics (IMCIC 2021) is to provide, in these increasingly related areas, a multi-disciplinary forum, to foster interdisciplinary communication among the participants, and to support the sharing process of diverse perspectives of the same transdisciplinary concepts and principles. Complexity, Cybernetics and Informatics are being increasingly related to each other in almost every scientific discipline, engineering area, and human activity. Their common transdisciplinarity characterizes and communicates them, generating strong relations among them and with other disciplines. They work together to create a whole new way of thinking and practice. This phenomenon persuaded the Organizing Committee to structure IMCIC 2021 as a multi-conference where participants may focus on one area, or on one discipline, while allowing them the possibility of attending events from other areas or disciplines. This systemic approach stimulates cross-fertilization among different disciplines, inspiring scholars, originating new hypothesis, supporting production of innovations and generating analogies; which is, after all, one of the very basic principles of the systems' movement and a fundamental aim in cybernetics.

One of the main objectives of the 12th International Conference on Society and Information Technologies (ICSIT 2021) and the 12th International Conference on Education, Training and Informatics (ICETI 2021) is to bring together academics, professionals, and managers from the private and the public sectors, so they can share ideas, results of research, and innovative services or products, in a multi-disciplinary and multi-sector forum.

Educational technologies, socio-economic organizations, and socio-political processes are essential domains among those involved in the evolving co-adaptation and co-transformation between societies and cultures on the one hand, and between informatics and cybernetics (communication and control) on the other hand.

ICSIT 2021 and ICETI 2021 are related to each other, as well as to IMCIC 2021 and, as a whole, are producing or might produce synergic relationships with Information and Communication Technologies. This is why the Organizing Committees have the purpose of combining their efforts in a way that would lead to the organization of an adequate joint event, where academics, researchers, consultants, professionals, innovators, and practitioners from the three areas might relate and interact with each other in the same event. These types of interaction might generate possibilities of cross-

fertilization and analogical thinking, as well as possibilities of new working hypothesis, ideas, and reflections on the impact, significance, and usefulness of Informatics and Cybernetics in important dimensions of educational, socio-political, and socio-economical processes, services, and products.

On behalf of the Organizing Committee, I extend our heartfelt thanks to the Program Committees' members, to the 495 reviewers, from 76 countries, who made the **double-blinded** reviews, and to the 175 reviewers, from 49 countries, who made the **non-blind** reviews. (Some reviewers supported both: non-blind and double-blind reviewing for different submissions). A total of 1148 reviews made by 670 reviewers (who made at least one review), from 82 countries contributed to the quality achieved in IMCIC/ICETI/CICIC 2021. This means an average of **4.43 reviews per submission** (259 submissions were received). Each registered author had access, via the conference web site, to the reviews that recommended the acceptance of their respective submissions. Each registered author could get information about: 1) the average of the reviewers' evaluations according to 8 criteria, and the average of a global evaluation of his/her submission; and 2) the comments and the constructive feedback made by the reviewers, who recommended the acceptance of his/her submission, so the author would be able to improve the final version of the paper.

In the organizational process of IMCIC/ICSIT 2021, about 221 articles were submitted. The IMCIC/ICSIT 2021 proceedings include about 87 papers from 34 countries (61 papers from IMCIC and 26 papers from ICSIT), which were accepted for presentation. *The submissions were reviewed as carefully as time permitted; it is expected that most of them will appear in a more polished and complete form in scientific journals. As announced in the conference web site* authors of the respective papers "are responsible of the **content** of their respective papers, as well as for their respective **proofreading and copyediting."**

This information is detailed and totalized in the following table, along with the other collocated event:

Conference	# of submissions received	# of reviewers that made at least one review	# of reviews made	Average of reviews per reviewer	Average of reviews per submission	# of papers included in the proceedings	% of submissions included in the proceedings
IMCIC 2021	139	287	453	1.58	3.26	61	43.88 %
ICSIT 2021	82	279	521	1.87	6.35	26	31.71 %
CICIC 2021	38	104	174	1.67	4.58	16	42.11 %
TOTAL 2021	**259**	**670**	**1148**	**1.71**	**4.43**	**103**	**39.77 %**

We also extend our gratitude to the co-editors of these proceedings, for the hard work, energy and eagerness they displayed in their respective activities. We express our intense gratitude to the late Professor William Lesso for his wise and opportune tutoring, for his eternal energy, integrity, and continuous support and advice, as the Program Committee Chair of past conferences, organized by the International Institute of Informatics and Systemics, as well as for being a very caring old friend and intellectual father to many of us.

Special thanks to Professors José Ferrer and Michael Savoie for co-chairing IMCIC 2021 Program Committee, to Professor Hsing-Wei Chu for his General Co-chairmanship, and to Belkis Sánchez for chairing its Organizing Committee. Special thanks also to Professor Friedrich Welsch for chairing

ICETI 2021, Professors José Ferrer and Belkis Sánchez for co-chairing its Organizing Committee, and to Professor Andrés Tremante for serving as General Co-chair.

We also extend our gratitude to the following scholars, researchers, and professionals who (up to the moment in which this Foreword is being written) accepted to deliver plenary Workshop and Tutorial, support the conference as panelists in conversational panels, chair conversational sessions and/or to address the audience of the General Joint Plenary Sessions with keynote addresses (More details, abstracts and short bios, were included in the Conference Program booklet and at: http://www.iiis.org/spring2021plenaryevents)

***Plenary Keynote Speakers** (in alphabetical Order)*

- **Dr. Francesco Armato**, University of Florence, Italy.
- **Dr. Risa Blair**, Purdue University Global, United States.
- **Ms. Sabrina Cesaretti**, University of Florence, Italy.
- **Dr. Areej Elsayary**, Zayed University, United Arab Emirates.
- **Dr. Nicola Fabiano**, Studio Legale Fabiano, Italy.
- **Professor Stefano Follesa**, University of Florence, Italy.
- **Professor T. Grandon Gill**, University of South Florida, United States.
- **Professor Shigehiro Hashimoto**, Kogakuin University, Japan.
- **Dr. Jeremy Horne**, Southwest Area Division- American Association for the Advancement of Science (President-Emeritus), United States.
- **Dr. Teresa Langness**, Full-Circle Learning, United States.
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- **Dr. Wayne Patterson**, Howard University (Retired Professor), United States.
- **Professor Ivan Semenist**, Borys Grinchenko Kyiv University, Ukraine.
- **Professor Allan Tarp**, MATHeCADEMY.net, Denmark.

We also would like to extend our appreciation and express our thankfulness and gratitude to the following invited session organizers, for their time and for getting in charge of the reviewing processes of their respective invited sessions.

Invited sessions Organizers (and Co-Organizers)

- **Professor Shigehiro Hashimoto**, Kogakuin University, Japan.
- **Professor Hirotoshi Hishida**, Kogakuin University, Japan.
- **Professor Nataļja Lāce,** Riga Technical University, Latvia.
- **Professor Areej ElSayary**, Zayed University, United Arab Emirates.

- **Professor Sufian Forawi,** The British University in Dubai, United Arab Emirates.

We wish to extend our gratefulness, as well, to those who accepted to host and moderate conversational sessions after proposing trans-disciplinary topics in order to foster inter-disciplinary communication, which a main founding purpose of the IIIS, and the reason for organizing the multi-disciplinary conferences.

Hosts and Moderators of the Conversational Sessions (Ordered According How They Were Scheduled in the Program)

- **Dr. Nicola Fabiano,** Founder and CEO of Studio Legale Fabiano, Italy.
- **Dr. Teresa Langness**, Nonprofit Board President at Full-Circle Learning, United States.
- **Dr. Jon K. Burmeister**, College of Mount St. Vincent, United States.
- **Dr. Ziyuan Meng**, Drew University, United States.
- **Professor Andrés Tremante**, Florida International University, United States.
- **Dr. Risa Blair**, Purdue University Global, United States.
- **Dr. Suzanne Lunsford**, Wright State University, United States.
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- **Professor Victoria E. Ospina Becerra**, Escuela Colombiana de Ingeniería Julio Garavito, Colombia.
- **Dr. Bertha Ulloa Rubio**, Universidad César Vallejo, Peru.
- **Dr. Eduardo A. Dutra Moresi**, Universidade Católica de Brasília, Brazil.

We also wish to thank all the authors for the quality of their papers, and to the 670 reviewers (who reviewed at least one article of IMCIC/ICSIT/CICIC 2021 and its collocated events) for the great job they did, making the 1148 reviews that supported the acceptance process. We also extend our gratitude to all the members of the secretariat and the staff for their knowledgeable effort in supporting the organizational process, the help desk, and the production of the proceedings.

Professor Nagib C. Callaos
IMCIC 2021 General Co-Chair

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Organizer: Natalja Lace (Latvia)

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ICT Applications in Social and Political Systems

Effect of Cell Type on Behavior of Cell under Shear Flow Field Before and After Division

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ABSTRACT

The effect of the cell type on orientation of each cell under the shear flow has been investigated *in vitro*. To make a Couette type of shear flow, the culture medium was sandwiched with a constant gap between parallel walls: a lower stationary culture disk, and an upper rotating disk. The wall shear stress (τ) on the lower culture disk was controlled by the rotating speed of the upper disk. Four types of cells were used in the test: C2C12 (mouse myoblast cell line), HUVEC (Human Umbilical Vein Endothelial Cells), 3T3-L1 (mouse fat precursor cells), and L929 (mouse fibroblast connective tissue). After cultivation without flow for 24 hours for adhesion of cells on the lower plate, the shear stress of 1 Pa was continuously applied on cells for 7 days in the incubator. The behavior (angle and deformation) of each cell (before and after division) was traced at the time lapse image observed by an inverted phase contrast microscope placed in an incubator. The experimental results show the following behavior of each cell: elongation and orientation under shear stress of 1 Pa depends on the cell type.

Keywords: Biomedical Engineering, Wall Shear Stress, Division, C2C12, HUVEC, L929, 3T3-L1 and Couette Flow.

1. INTRODUCTION

A biological cell migrates on the scaffold. In several cases, cells are exposed to the shear stress both *in vivo* and *in vitro*. The direction of the shear stress field might affect the direction of the migration. The hysteresis also might affect the direction of the migration. The behavior of each cell might depend on the initial state. In the most of tests *in vitro*, cells are incubated for several hours for adhesion to the scaffold before flow stimulation. Each cell can make division under the wall shear stress field. Is the effect maintained in each cell after division? The adhesion status of the cell is controlled by itself after division. In the present study, the migration of each cell is tracked at the division in the shear stress field of the medium.

The effect of the shear flow on the endothelial cells, which are exposed to the blood flow on the inner surface of the vessel wall, were investigated in many studies [1-5]. In the previous study with the vortex flow by the swinging plate in vitro, C2C12 made orientation perpendicular to the direction of the flow, although HUVEC made orientation along the streamline of the flow [6]. The orientation of each cell in the tissue depends on that of neighbor cell.

At the wall shear stress, a cell might show the following responses: elongation [3], tilting to the streamline [6], migration [4], deformation to be rounded, proliferation [5], and exfoliation from the wall of the scaffold. In the Poiseuille type of flow, the shear rate depends on the distance from the wall: highest at the wall. In the Couette type of flow, on the other hand, the shear rate is constant regardless of the distance from the wall.

In the present study, an experimental system of the Couette type flow in the constant gap with a rotating disk has been designed to apply the shear stress quantitatively on the cell during incubation at the microscopic observation in vitro, and the effect of the shear stress field (1 Pa) on each cell at division has been studied about migration.

2. METHODS

Shear Flow Device
To apply the constant shear flow field to the cell culture, a Couette type of shear flow device has been used (Fig. 1). The shear field is generated between a rotating disk and a stationary dish. The medium is sheared between a rotating wall and a stationary wall. The stationary wall is the bottom of the culture dish (diameter 60 mm).

In the device, the shear rate (γ) in the medium is calculated by Eq. (1).

$$\gamma = r\,\omega / d \tag{1}$$

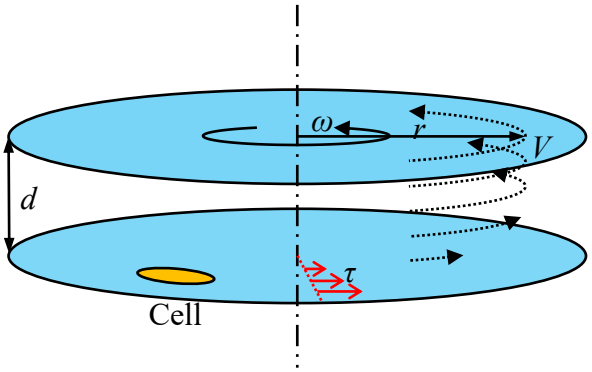

Fig. 1: Couette flow (velocity (V) distribution) between rotating (angular velocity ω) wall and stationary wall at r (radius) (distance d): wall shear stress (τ).

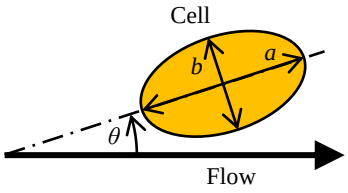

Fig. 2: Shape index and angle of cell exposed to flow.

In Eq. (1), ω is the angular velocity [rad/s], and d is the gap [m] between the wall of the moving disk and the wall of stationary plate. Between the parallel walls, d is constant. The shear rate (γ [s^{-1}]) in the gap between walls increases in proportion to the distance (r [m]) from the axis of the rotation. The angular velocity ω (< 22 rad/s) was controlled by the stepping motor. In the observation area of the microscope, r varies between 17 mm and 18 mm. The distance d, which was measured by the positions of the focus of the walls at the microscope, was around 0.6 mm. The shear rates (γ) of 6.7×10^2 s^{-1} are made in the present experiment by adjustment of these parameters.

The shear stress (τ [Pa]) is calculated by the viscosity (η [Pa s]) of the medium.

$$\tau = \eta\,\gamma \tag{2}$$

Using the viscosity of the medium of 1.5×10^{-3} Pa s (measured by a cone and plate viscometer at 310 K), the shear stress τ have been calculated as the value of 1 Pa. The rotating disk device is mounted on the stage of the inverted phase contrast microscope placed in the incubator. The device allows the microscopic observation of cells cultured on the stationary wall during exposure to the shear flow field.

Cell Culture

Four types of cells were used in the test: C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse, passage between eight and ten), HUVEC (Human Umbilical Vein Endothelial Cells), 3T3-L1 (mouse fat precursor cells, a cell line derived from cells of mouse 3T3), and L929 (fibroblast connective tissue of C3H mouse). Cells were cultured in D-MEM (Dulbecco's Modified Eagle's Medium): containing 10% decomplemented FBS (fetal bovine serum), and 1% penicillin/ streptomycin.

The cells were seeded on the dish at the density of 3000 cells/cm^2. To make adhesion of cells to the bottom of the culture dish, the cells were cultured for 24 hours in the incubator without flow stimulation (without rotation of the disk). After the pre-incubation for 24 hours without shear, the cells were continuously sheared with the rotating disk for 24 hours in the incubator at the constant rotating speed. The constant speed was preset for each test to keep the designed shear stress field.

Image Analysis

The time-lapse microscopic image was taken every ten minutes during the cultivation. The contour of each cell adhered on the stationary plate of the scaffold was traced. The contour of each cell was approximated to ellipsoid. From these images, cells with the division were picked up. At each cell, data were traced before and after two hours from the division.

On the ellipsoid, the length of the major axis (a), and the length of the minor axis (b) were measured. The ratio of axes is calculated as the shape index (P) by Eq. (3).

$$P = 1 - b / a \tag{3}$$

At the circle, P = 0. As the ellipsoid is elongated, P approaches to one. The acute angle (θ) between the major axis and the flow direction was measured (Fig. 2).

3. RESULTS

Fig. 3 exemplifies data of C2C12. Fig. 4 exemplifies data of HUVEC. Fig. 5 exemplifies data of 3T3-L1. Fig. 6 exemplifies data of L929. Figs. 3a, 4a, 5a and 6a show the shape index P at control (without the shear field). Figs. 3b, 4b, 5b and 6b show the shape index P exposed to the shear field. Figs. 3c, 4c, 5c and 6c show the angle θ at control (without shear flow). Figs. 3d, 4d, 5d and 6d show the angle θ exposed to the shear field. The same symbol marker shows the same cell tracked after division, respectively. The deformation of HUVEC is larger than that of the other cell type. The shape index decreases much before division, and increases much after division. The deformation of C2C12 is smaller than that of the other cell type. 3T3-L1 with higher shape index tends to tilt to the flow direction.

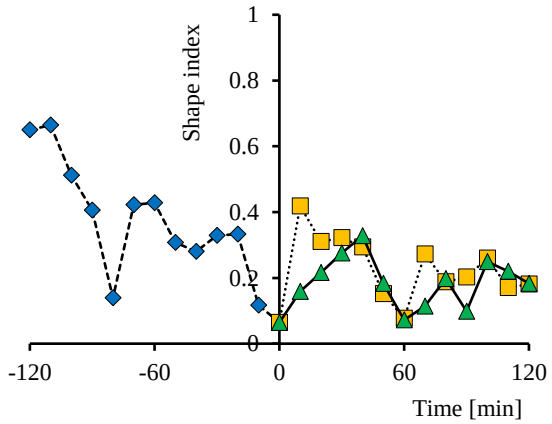

Fig. 3a: Shape Index before and after division: C2C12: control.

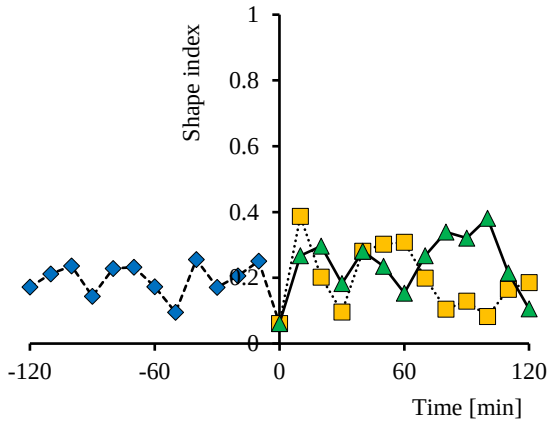

Fig. 3b: Shape Index before and after division: C2C12: 1 Pa.

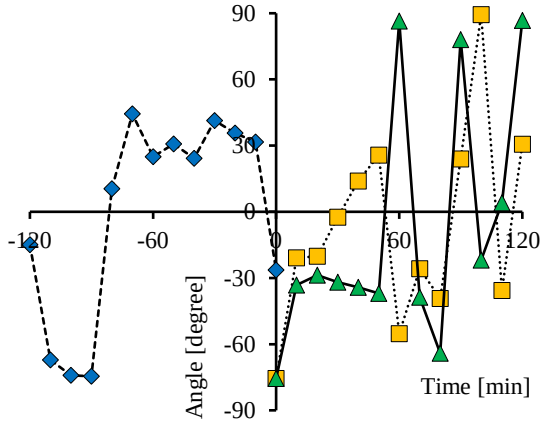

Fig. 3c: Angle before and after division: C2C12: control.

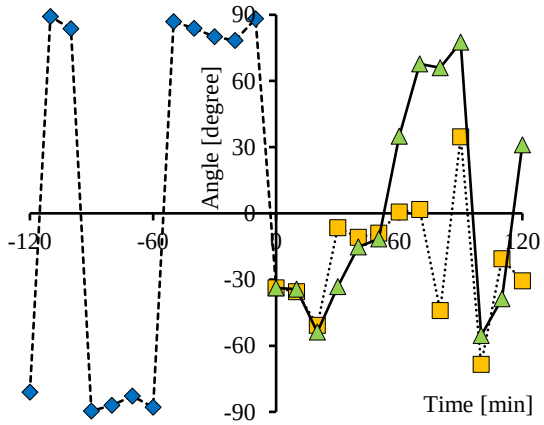

Fig. 3d: Angle before and after division: C2C12: 1 Pa.

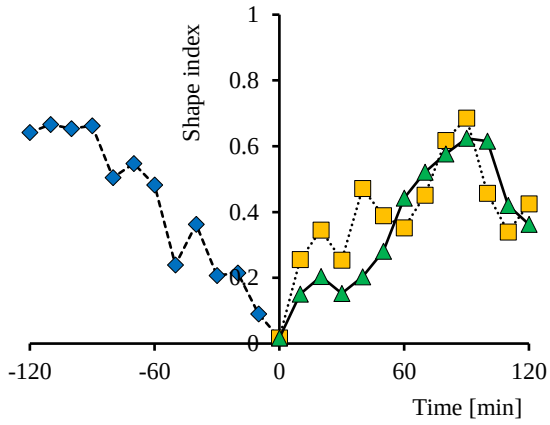

Fig. 4a: Shape Index before and after division: HUVEC: control.

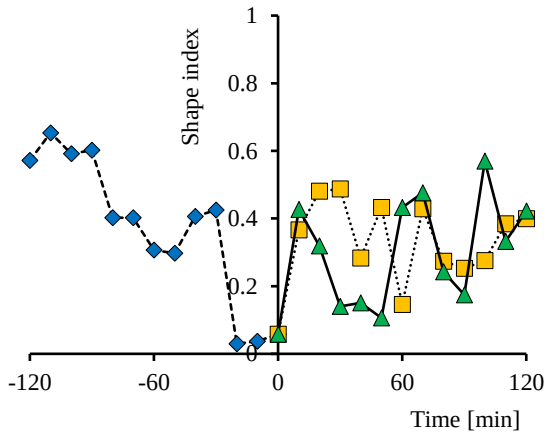

Fig. 4b: Shape Index before and after division: HUVEC: 0.9 Pa.

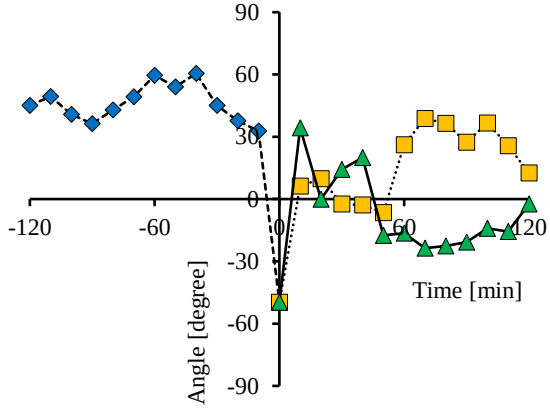

Fig. 4c: Angle before and after division: HUVEC: control.

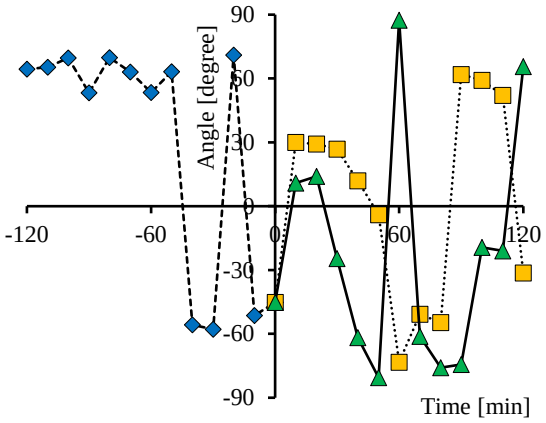

Fig. 4d: Angle before and after division: HUVEC: 0.9 Pa.

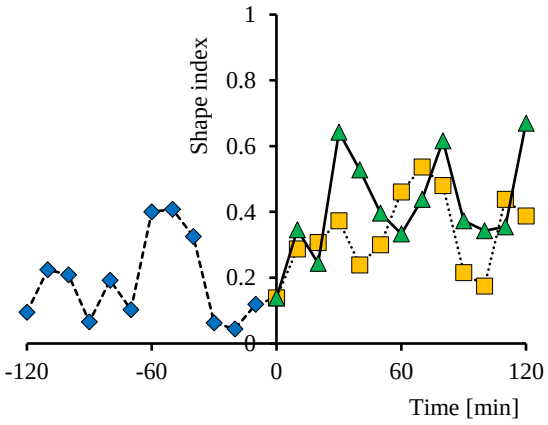

Fig. 5a: Shape Index before and after division: 3T3-L1: control.

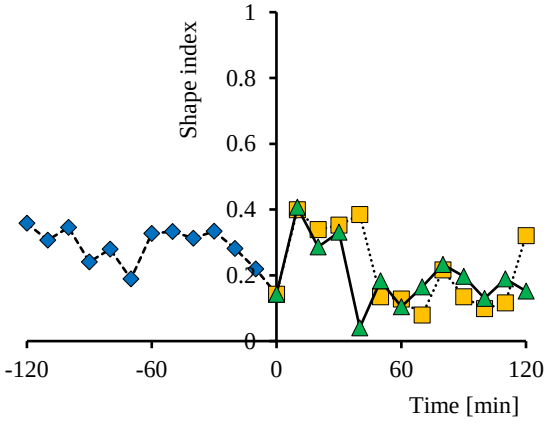

Fig. 5b: Shape Index before and after division: 3T3-L1: 1 Pa.

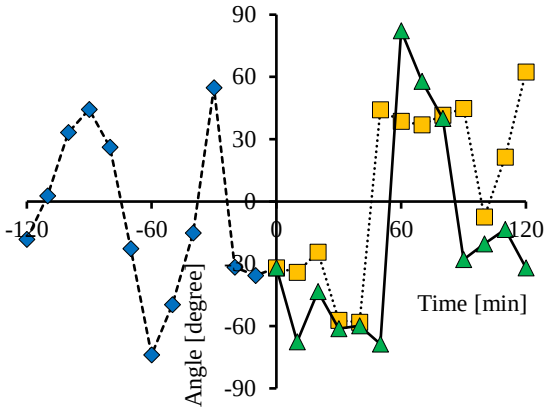

Fig. 5c: Angle before and after division: 3T3-L1: control.

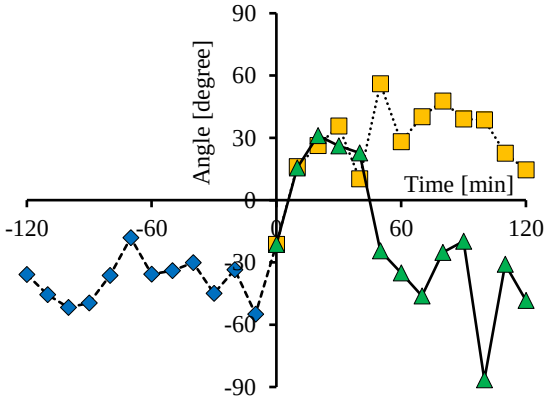

Fig. 5d: Angle before and after division: 3T3-L1: 1 Pa.

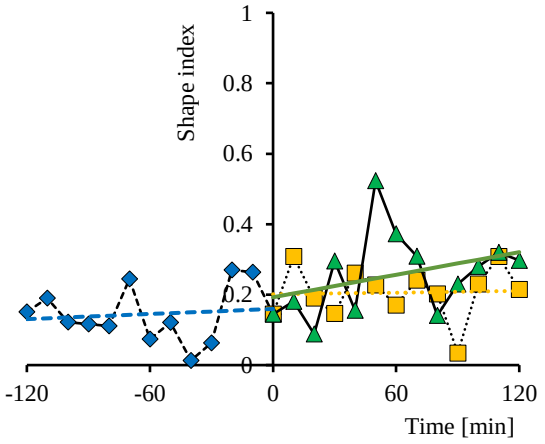

Fig. 6a: Shape Index before and after division: L929: control.

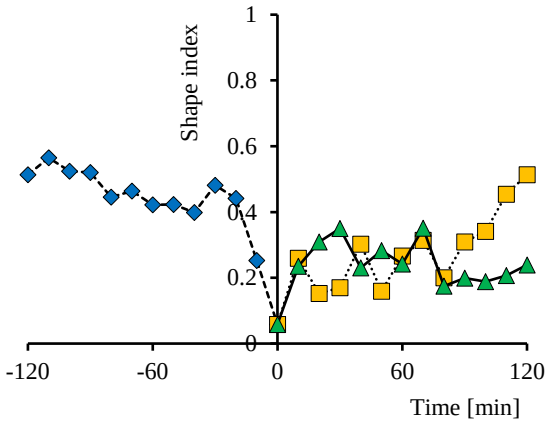

Fig. 6b: Shape Index before and after division: L929: 1 Pa.

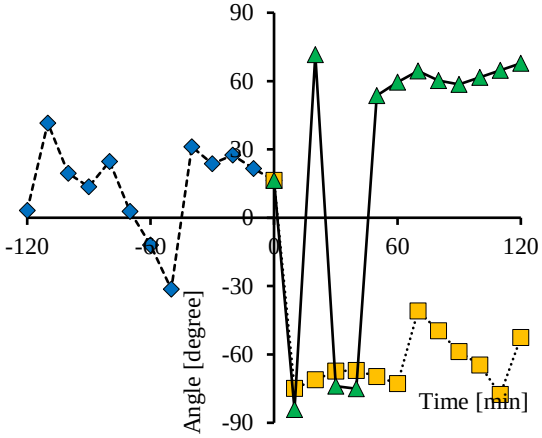

Fig. 6c: Angle before and after division: L929: control.

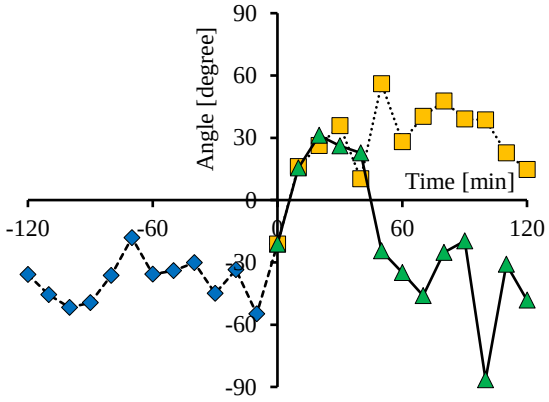

Fig. 6d: Angle before and after division: L929: 1 Pa.

Figs. 7a and 7b show the relationship between the angles before and after division. Fig. 7a shows data of C2C12. The regression line (the correlation coefficient of 0.73) shows the positive slope. After division, C2C12 tends to return to the same direction as that of before division under the shear stress field of 1 Pa. Fig. 7b shows data of 3T3-L1. The regression line (the correlation coefficient of −0.42) shows the negative slope. After division, 3T3-L1 tends to change the direction different from that of before division under the shear stress field of 1 Pa.

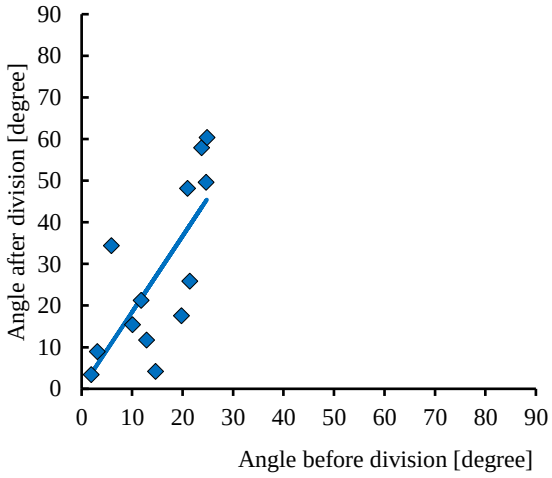

Fig. 7a: Angle before and after division: C2C12: 1 Pa: $r = 0.73$.

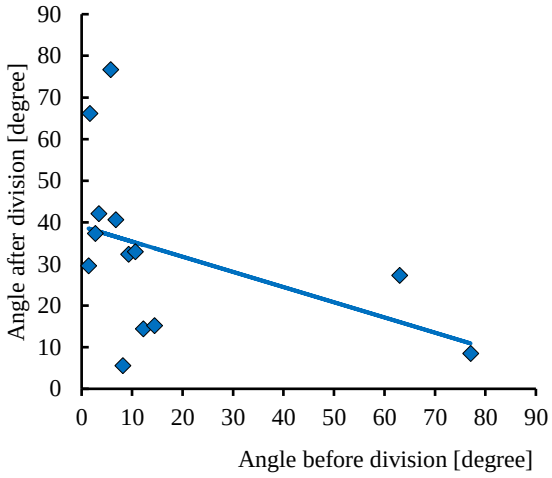

Fig. 7b: Angle before and after division: 3T3-L1: 1 Pa: $r = -0.42$.

4. DISCUSSION

A biological cell shows passive and active responses in an environment. While the flow enhances the cell migration to the downstream, a cell migrates to adapt to the shear field. While the strong stimulation above the threshold damages the cell, the stimulation below the threshold remains in the cell as a memory for the response in the next step [7]. The hysteresis effect governs the active response of the cell. In the previous study, cells were exposed to the shear flow in a donut-shaped open channel, and the effect of flow stimulation on cultured cells has been studied in vitro [6]. When the flow has an open surface, it is difficult to estimate the shear stress value in the fluid. Between two parallel walls, on the other hand, the velocity profile is estimated to be parabolic in the laminar flow. In the previous studies, several preparations were designed to study the effect of mechanical flow stimulations on biological cells: the tilting disk channel, the rhombus channel, the crossflow channel, and the rotating disk type.

The Couette type of flow is convenient to estimate the shear stress in the flow with the constant shear rate between the moving wall and the stationary wall, which is also available to non-Newtonian fluid. Several kinds of the devices of Couette type flow were designed for quantitative experiments of biological

fluid in the previous studies [8-10]. The cone-and-plate type device has the uniform shear field in the entire space between the rotating cone and the stationary plate. The shear stress is constant independent of the distance from the rotating axis. The erythrocyte destruction was studied between the rotating concave cone and the stationary convex cone. A parallel disks system between rotating disk and the stationary disk, on the other hand, has several advantages: stability of the rotating motion of the disk, stability of the optical path for the microscopic observation, morphologic preciseness of the plane of the disks, and simultaneous observation over the range of variation of the shear rate proportional to the radius from the rotational axis. The floating erythrocyte deformation was observed between counter rotating parallel discs. The steady actual flow direction adjacent to the scaffold surface of cell culture has been confirmed by the streamline traced by the direction of exfoliation of the cell and of the moving particle adjacent to the surface [1]. The flow velocity, which increases in proportional to the distance from the rotating axis, has also been confirmed by tracings of the moving particle adjacent to the surface.

The most of myoblasts tend to migrate to the oblique direction of the lower shear stress field at 1 Pa. The effect of shear flow on cells depends on the cell types. The dependency might be applied to the cell sorting technology [8]. After division, cells tend to migrate counter direction each other. The tendency makes symmetrical distribution of the migration velocity of cells in Figs. 6b and 7b. To trace the cell after division is convenient to study on the initial behavior of the cell. The cells proliferate regardless of the shear flow stimulation. The cell cycle does not vary under the shear flow. The movement of each cell can be traced by the time lapse images with the interval of ten minutes in the present experiment. The effect of shear flow on cells was investigated in many previous studies. The shear flow affects adhesion of each cell [11]. Adhesion of cells can be controlled with design of the scaffold [12]. The behavior of cells in the shear flow was simulated in the previous study [13]. The effect of fluid induced shear stresses on osteoblasts was studied in the previous study [14].

5. CONCLUSIONS

The experimental results show the following behavior of each cell type. The deformation of HUVEC is larger than that of the other cell type. The shape index decreases much before division, and increases much after division. The deformation of C2C12 is smaller than that of the other cell type. After division, C2C12 tends to return to the same direction as that of before division under the shear stress field of 1 Pa. After division, 3T3-L1 tends to change the deformation direction different from that of before division under the shear stress field of 1 Pa.

ACKNOWLEDGMENT

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Behavior of Cell on Micro Ridge Pattern After Continuous Stimulation of Tangential Force

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ABSTRACT

The behavior of each cell on the micro ridge after the continuous stimulation of the tangential force has been studied *in vitro*. The stripe-pattern (0.7 μm height, 3 μm width, and 3 μm interval) was made on the surface of the scaffold plate to control the orientation of each cell during the force field stimulation. Variation was made on the angle between the longitudinal direction of the ridge and the direction of the tangential force: 0 degree, 45 degrees, and 90 degrees. Myoblast (C2C12: mouse myoblast cell line) was used in the experiment. To apply the tangential force field to the cells, the scaffold plate was set in a tube in a conventional centrifugal machine placed in an incubator. After the centrifugation for 5 hours, the behavior of each cell at the time-lapse microscope images was traced for 10 hours. The experimental results show that cells on the ridge-lines perpendicular to the tangential force field have the higher activity (migration, and deformation) after stimulation.

Keywords: Biomedical Engineering, Cell Culture, Tangential Force Field, Division and C2C12.

1. INTRODUCTION

The recent cell culture technique enables observation of the behavior of each cell *in vitro*. A biological cell adheres on the scaffold and shows several active behaviors: migration, deformation, and proliferation. The cell is deformed by the force, because of its compliance. The cell deforms, on the other hand, to minimize the intra force of itself. The cell is moved by the force. The cell, on the other hand, moves by itself. C2C12 is cell line of mouse myoblast. In the previous study, both the migration and the deformation of C2C12 were restricted by the wall shear stress higher than 3 Pa in flow *in vitro* [1]. Deformability of C2C12 was observed by the slit in the previous study *in vitro* [2, 3]. C2C12 extended along the lines of micro ridges on the scaffold surface [4]. Vibration decelerated adhesion of C2C12 on the micro ridges [5]. C2C12 migrated regardless of the direction of the vibrating micro ridges. The orientation of C2C12 was tried to be measured by the electric impedance *in vitro* [6]. Both differentiation and growth of C2C12 were delayed with electric pulses in the previous study *in vitro* [7]. The floating cells of C2C12 were tried to be sorted by the micro groove on the wall of the flow channel [8].

These behaviors might depend on the history of each cell. While the strong stimulation above the threshold damages the cell, the stimulation below the threshold remains in the cell as a memory for the response in the next step. The memory might be reset after division of the cell. To trace the hysteresis effect of the mechanical stimulation on the single cell, the time-lapse images are effective. In the previous study, the longitudinal axis of C2C12 (mouse myoblast cell line) tends to align to the direction of the excess gravity. The axis tilts to the perpendicular direction, on the other hand, after stopping of the excess gravity [9]. A single cell migrates at random on the scaffold. The cell tends to align to the longitudinal direction of the micro ridge line [10].

In the present study, the behavior of each single cell on the micro ridge pattern after the exposure to the tangential force field by centrifuge has been investigated *in vitro*.

2. METHODS

Micro-Ridge pattern on Scaffold Plate
To control the orientation of each cell, the micro striped pattern has been made in three partial rectangular areas of 0.4 mm × 1.6 mm on the PDMS (polydimethylsiloxane) plate of the scaffold by photolithography technique. The height, the width, and the interval of the quadrangular ridges are 0.7 μm, 3 μm, and 3 μm, respectively. Each area has its own specific direction of the striped pattern. Namely, variation has been made on the angle (α) between the longitudinal direction of the ridge and the direction of the tangential force: 0 degree (parallel), 45 degree, and 90 degree (perpendicular). Three partial area was made on the same surface of the scaffold plate in parallel position, so that the behavior of cells on each area can be compared simultaneously. The pattern of each area was also used as a marker to trace each cell.

Mold for Micro Ridge Pattern

The borosilicate glass (Tempax) disk was used for the base of the mold of the micromachining process. The positive photoresist material of OFPR-800 (Tokyo Ohka Kogyo Co., Ltd, Tokyo, Japan) was coated on the glass with the spin coater (at 3000 rpm for 20 s). The micro pattern was drawn on the mold with a laser drawing system (DDB-201K-KH, Neoark Corporation, Hachioji, Japan). The photoresist was developed with tetra-methyl-ammonium hydroxide (NMD-3, Tokyo Ohka Kogyo Co., Ltd., Kawasaki, Japan) for two minutes. The glass plate with the photoresist material was etched with the plasma gas using RIE-10NR (Samco International, Kyoto, Japan).

After the mold of the glass disk was enclosed with a peripheral wall of polyimide tape, PDMS (Sylgard 184 Silicone Elastomer Base, Dow Corning Corp., MI, USA) was poured with the curing agent (Sylgard 184 Silicone Elastomer Curing Agent, Dow Corning Corp., MI, USA) on the mold. The volume ratio of PDMS to curing agent is ten to one. The baked plate of PDMS (1 mm thickness) was exfoliated from the mold of the slide glass plate. The PDMS plate was cut to make a plate of 15 mm × 10 mm × 1 mm, and stacked on the glass plate of 50 mm × 13 mm × 1 mm. To make the surface hydrophilic, the surface of the PDMS plate was exposed to the oxygen gas (0.1 Pa, 30 cm^3/min) in the reactive ion etching system (FA-1: oxygen plasma ashing, 50 W) for thirty seconds just before the cell culture.

Tangential Force

The tangential force field was applied to the culture surface with the centrifugal force. The culture plate is inserted into the tube, which is contained in the rotor. The glass plate was set so that the culture surface is parallel to the radial direction of the rotation. The centrifugal force is applied parallel to the culture surface. The centrifugal force (Fc) is calculated by Eq. (1).

$$Fc = m\, r\, \omega^2 \tag{1}$$

In Eq. (1), m is mass, r is radius of the rotation, and ω is angular velocity. In the gravitational field, gravitational force (Fg) is calculated by Eq. (2), where g is gravitational acceleration.

$$Fg = m\, g \tag{2}$$

In the present study, the centrifugal acceleration lower than 100 G (1 G is equal to the gravitational acceleration) ((Fc / Fg) < 100) is applied with the centrifugal machine.

Cell Culture

C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse) was used in the test. C2C12 of the passage four was cultured in D-MEM (Dulbecco's Modified Eagle's Medium) containing 10% decomplemented FBS (fetal bovine serum) and 1% penicillin/ streptomycin. Cells were seeded on the glass plate at the density of 3000 cells/cm^2. After the cells were cultured for 12 hours in the resting state, the excess gravitational stimulation was applied for five hours. To apply the tangential force field to the cells, the scaffold plate was dipped in the medium in a tube in a conventional centrifugal machine (CN-1040, Matsuura seisakusyo. Ltd, Tokyo, Japan) placed in an incubator. Several tubes with the glass plate were set in the rotor of the centrifugation to cultivate cells, simultaneously. To keep the content of carbon dioxide of 5 % at 310 K, the cells were cultured in an incubator through the entire experimental term including the term of exposure to the tangential force field. Namely, the centrifugal machine was placed in the incubator during the centrifugation.

Image Analysis

After stimulation of the tangential force for 5 hours, cells on the glass plate were moved from the centrifugal tube to the culture dish. Cells were subsequently cultured in the dish placed in the incubator combined with an inverted phase-contrast microscope (LCV110-SK, Olympus Co., Ltd., Tokyo). The behavior of each cell was analyzed on the time-lapse images captured every ten minutes for 48 hours after stimulation of the tangential force. "Image J" was applied to analyze the behavior of each cell. On the microscopic image, the contour of each cell was traced. The contour except pseudopodia was approximated to ellipsoid (Fig. 1). The centroid is used to trace the migration of each cell. The velocity (v [μm/min]) of the migration of each cell was calculated by Eq. (3) using the distance (Δx) between the centroids of the moving cell at the time-lapse microscopic images (at t_1 and at t_2) with the interval of ten minutes (Fig. 2).

$$v = \Delta x / (t_2 - t_1) \tag{3}$$

The area change rate (Sr [%/min]) was calculated by Eq. (4) using two-dimensional projected area of each cell at the time-lapse microscopic images with the interval of ten minutes.

$$Sr = 100 \times (S_2 - S_1) / (S_1 \times (t_2 - t_1)) \tag{4}$$

In Eq. (4), S_1 and S_2 are the areas of the tracked cell at the time t_1 [min] and at the time t_2 [min], respectively.

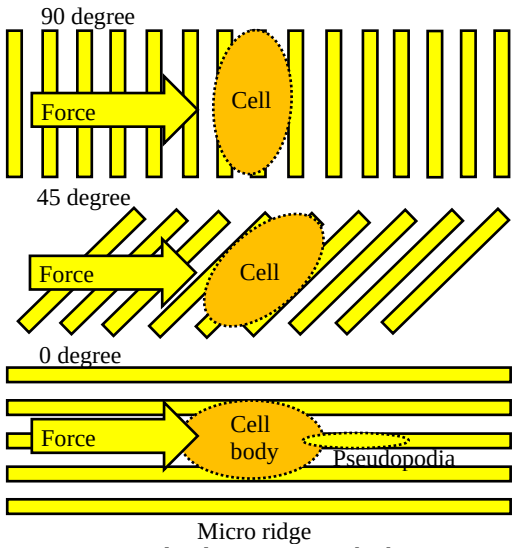

Fig. 1: Micro striped ridge pattern with three variations of direction against tangential force field: 90 degree, 45 degree, and 0 degree.

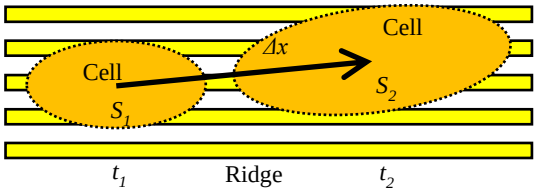

Fig. 2: Migration (Δx) and deformation (from S_1 to S_2) of cell on pattern.

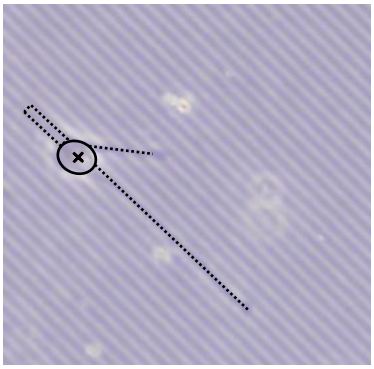

Fig. 3: Contour of cell except pseudopodia (dotted lines) was approximated to ellipsoid (solid line): centroid (cross mark).

3. RESULTS

Each cell extended pseudo to the direction of the longitudinal direction of the micro ridge (Fig. 3). Each cell deformed along to the longitudinal direction of the micro ridge. Each cell migrated along the longitudinal direction of the micro ridge, as the longitudinal axis of each cell was parallel to that of the micro ridge.

The following figures show the results on the surface of variations of the direction of the striped pattern: no pattern (Figs. 4a, 5a, 6a, and 7a), 0 degree (parallel to the tangential force; Figs. 4b, 5b, 6b, and 7b), 45 degrees (Figs. 4c, 5c, 6c, and 7c), and 90 degrees (perpendicular to the tangential force; Figs. 4d, 5d, 6d, and 7d), respectively. Figs. 4 and 6 show the behavior of cells for 10 hours after 5 hours exposure to the tangential force field of 50 G. Figs. 5 and 7 show the behavior of cells for 10 hours after 5 hours exposure to the tangential force field of 100 G.

Each marker corresponds to each cell tracked at the time lapse images. In Figs. 4d, 6d, and 8d, cell 1 (rhombus), cell 1.1 (square), cell 1.2 (triangle), cell 2 (cross, $S = 69\,v + 318$, $r = 0.52$), and cell 3 (circle). The cell 1 was divided into the cell 1.1 and the cell 1.2. In Figs. 4a and 6a, cell 7 (open circle), cell 8 (cross), cell 8.1 (square), cell 8.2 (closed circle), cell 8.2.1 (triangle), and cell 8.2.2 (rhombus). The cell 8 was divided into the cell 8.1 and the cell 8.2. The cell 8.2 was divided into the cell 8.2.1 and the cell 8.2.2. In some cases, the regression line of each cell tracking is displayed in the figures. In the other cases, the correlation coefficient (r) at each regression line is lower than 0.35.

Fig. 4 shows the tracing of migration velocity of each cell for 10 hours after 5 hours exposure to the tangential force field of 50 G. Fig. 5 shows the tracing of migration velocity of each cell for 10 hours after 5 hours exposure to the tangential force field of 100 G. The migration velocity of each cell on the striped pattern of micro ridge perpendicular to the tangential force tends to increase after the tangential force stimulation (Figs. 4d and 5d). On the striped pattern of micro ridge parallel to the tangential force, on the other hand, the migration velocity is relatively lower (Figs. 4b and 5b). The tendency is remarkable after centrifuge at 50 G than 100 G. The migration velocity also increases before and after the deviation of the cell (Fig. 4a). Data of the cell on the flat surface without the striped pattern of micro ridge fluctuate. The longitudinal direction of each cell changes at random on the flat surface.

Figs. 6 and 7 shows the area change rate (Sr) tracing of each cell for 10 hours. The change rate of each cell contact area to the scaffold tends to be higher on the striped pattern of micro ridge perpendicular to the tangential force (Fig. 6d). On the striped pattern of micro ridge parallel to the tangential force, on the other hand, the change rate of each cell contact area to the scaffold tends to be lower (Fig. 6b). The tendency is remarkable after centrifuge at 50 G than 100 G. The higher change rate neutralized with time, after the stimulation of the tangential force field. The change rate of the cell contact area to the scaffold is high, after the division of the cell (Figs. 6a and 6d).

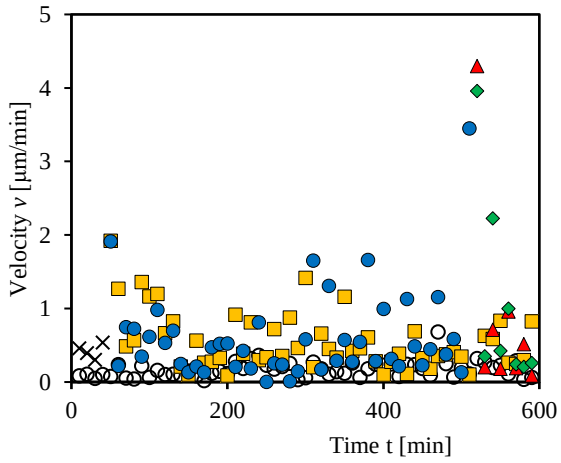

Fig. 4a: Velocity tracings of cell migration (50 G, control).

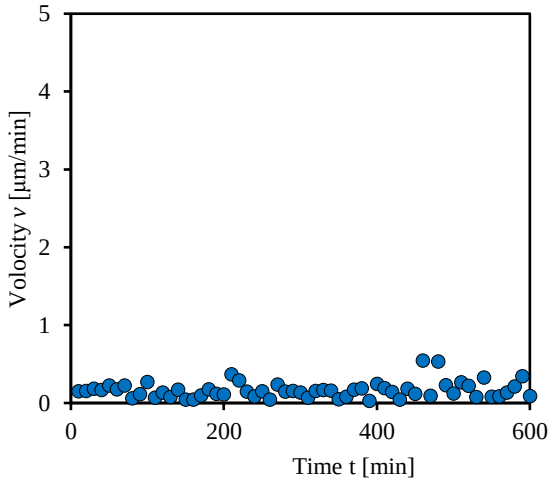

Fig. 4b: Velocity tracings of cell migration (50 G, 0 degree).

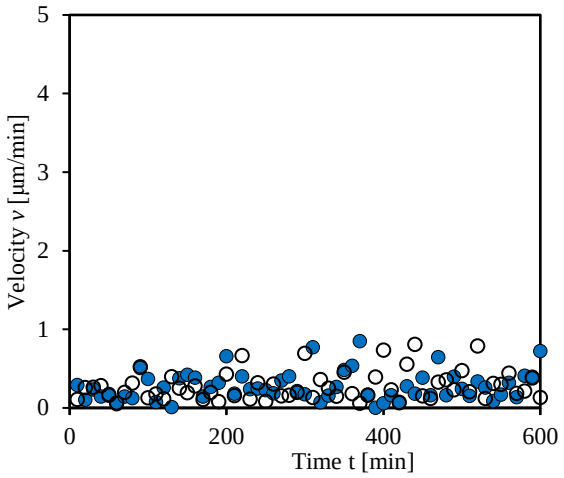

Fig. 4c: Velocity tracings of cell migration (50 G, 45 degree).

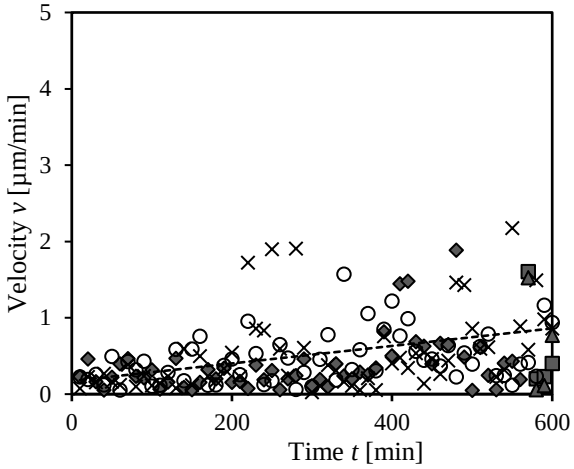

Fig. 4d: Velocity tracings of cell migration (50 G, 90 degree): cross, broken line, $v = 0.0011\ t + 0.17$, $r = 0.39$.

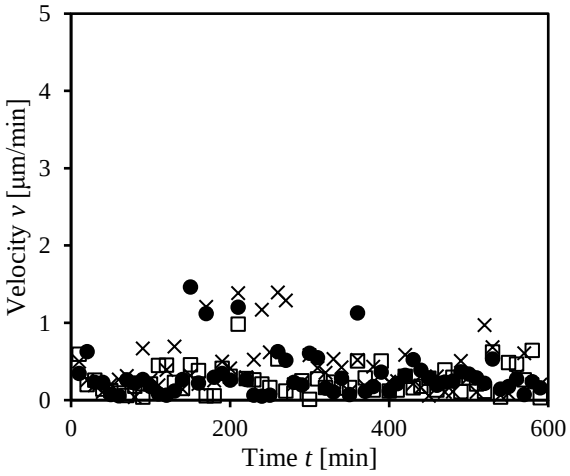

Fig. 5a: Velocity tracings of cell migration (100 G, control).

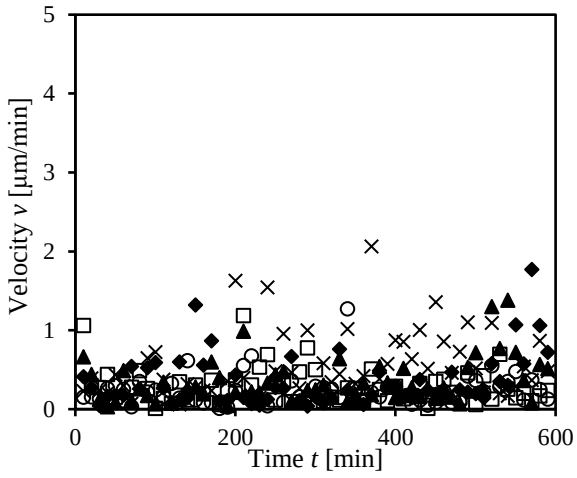

Fig. 5b: Velocity tracings of cell migration (100 G, 0 degree).

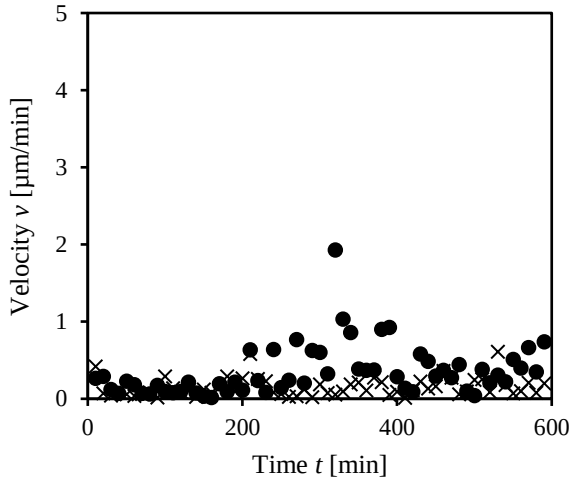

Fig. 5c: Velocity tracings of cell migration (100 G, 45 degree).

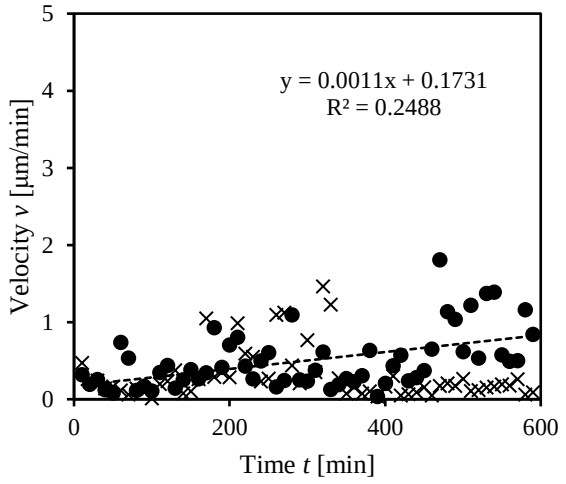

Fig. 5d: Velocity tracings of cell migration (100 G, 90 degree): circle, broken line, $v = 0.0011\ t + 0.17$, $r = 0.50$.

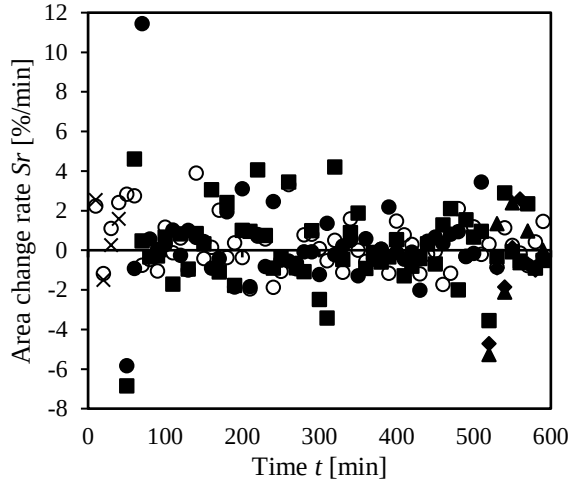

Fig. 6a: Tracings of area change rate (50 G, control).

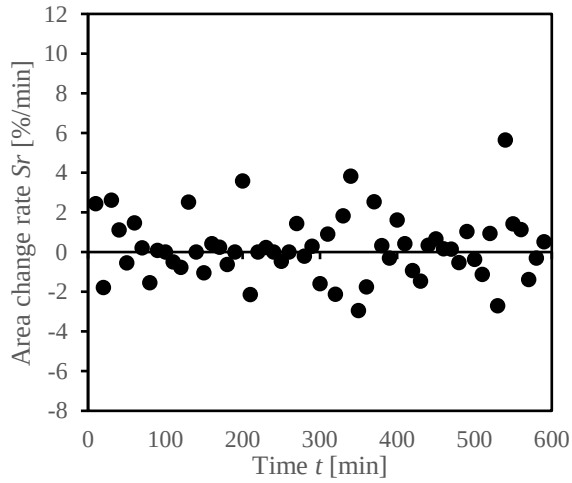

Fig. 6b: Tracings of area change rate (50 G, 0 degree).

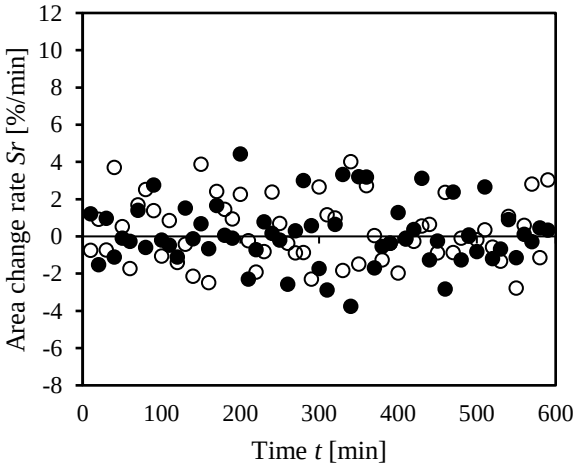

Fig. 6c: Tracings of area change rate (50 G, 45 degree).

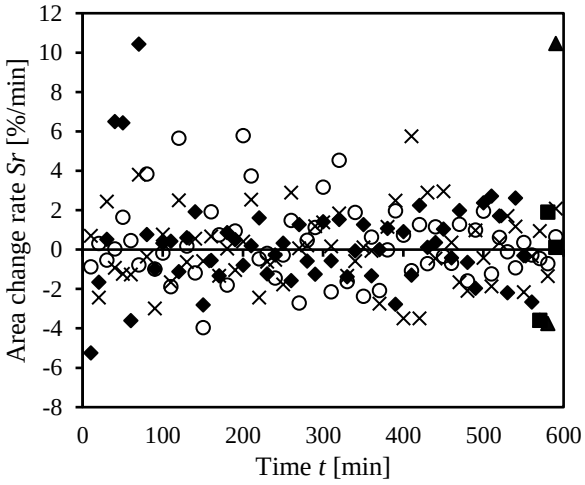

Fig. 6d: Tracings of area change rate (50 G, 90 degree).

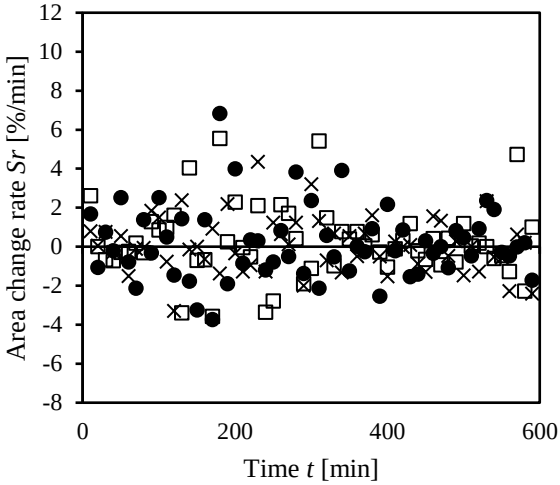

Fig. 7a: Tracings of area change rate (100 G, control).

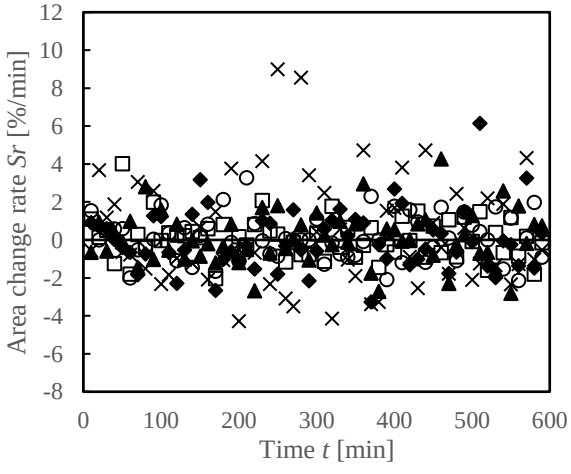

Fig. 7b: Tracings of area change rate (100 G, 0 degree).

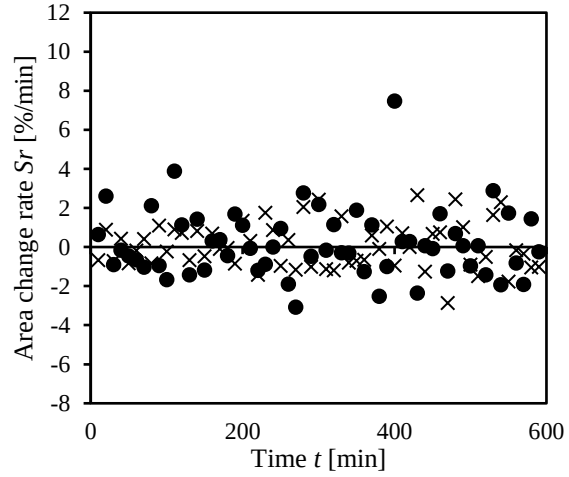

Fig. 7c: Tracings of area change rate (100 G, 45 degree).

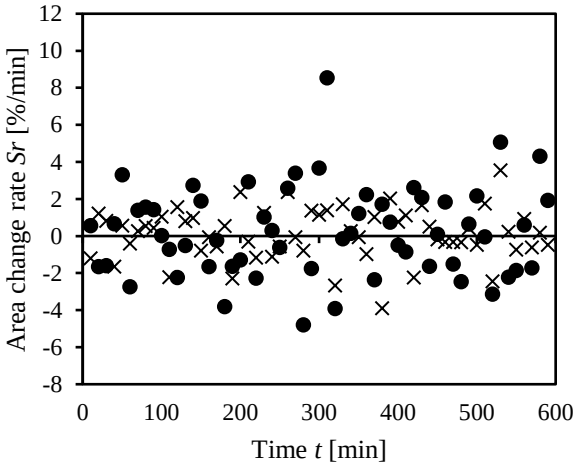

Fig. 7d: Tracings of area change rate (100 G, 90 degree).

4. DISCUSSION

The hysteresis effect of the tangential force stimulation on the activity of each cell was reset after division in the previous study. The gravity in the fluid is reduced by the buoyancy. Measurement of the density of cells by Phthalate ester method shows that the mean density of each cell is 1.07×10^3 kg/m^3. When the cells floating in the medium of the density of 1.00×10^3 kg/m^3, the effective centrifugal force ratio calculated from the difference of two density is 7 G at centrifuge of 100 G.

The apparent area of the cell contact to the scaffold was approximated to the two-dimensional projected area of the microscopic image in the present study. The three-dimensional contact area depends on the surface micro-topography. The special attention is necessary that the chemical property of the interface also affects the real contact area between the cell and the scaffold.

The height of the ridges was limited to 0.7 μm, which is minimum value to control the alignment of each cell [10]. In the present study, the striped pattern of the micro ridges is effective to control the longitudinal direction of each cell.

5. CONCLUSIONS

The experimental results show that the activity of the cell (migration, and deformation) after stimulation is maximized on the stripe pattern perpendicular to the tangential force field.

ACKNOWLEDGMENT

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Effect of Activity of Cell on Division under Shear Flow Field

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ABSTRACT

The effect of activity of the cell on division has been investigated under the shear flow field *in vitro*. To make a Couette type of the shear flow, the culture medium was sandwiched with a constant gap between parallel disks: a lower stationary culture disk, and an upper rotating disk. The wall shear stress (τ) on the inner surface of the lower culture plate was controlled by the rotating speed of the upper disk. Myoblasts (C2C12: mouse myoblast cell line) were used in the test. After cultivation without flow for 24 hours for adhesion of cells on the lower plate, τ was continuously applied on cells for 7 days in the incubator. The behavior of each cell was traced at the time-lapse image observed by an inverted phase contrast microscope placed in an incubator. The experimental results show that a cell tends to make division after the higher activity: deformation, and migration. Each cell tends to become active before and after division. A cell tends to be exfoliated after the lower activity.

Keywords: Biomedical Engineering, C2C12, Deformation, Migration, Wall Shear Stress and Division.

1. INTRODUCTION

The effect of the shear flow on the endothelial cells, which are exposed to the blood flow on the inner surface of the vessel wall, were investigated in many studies [1–10]. In the previous study with the vortex flow [11] by the swinging plate *in vitro*, C2C12 (mouse myoblast cell line) made orientation perpendicular to the direction of the flow, although HUVEC (human umbilical vein endothelial cell) made orientation along the streamline of the flow [12]. The orientation of each cell in the tissue depends on that of neighbor's cell. To analyze the mechanism of making orientation of cells, the behavior of each cell in the shear flow field should be quantitatively observed during tissue making.

At the wall shear stress, a cell shows the following responses: deformation [1–3], rotation [4, 5], migration [6, 7], division [7, 8, 13], exfoliation [14–16], and fusion [17]. In the Poiseuille type of flow, the shear rate depends on the distance from the wall: highest at the wall [14–16, 18]. In the Couette type of flow, on the other hand, the shear rate is constant regardless of the distance from the wall [17, 19, 20]. It takes several hours for adhesion of cells to the scaffold. The effect of the shear flow depends on the alignment of the cell. At division [21] as well as at fusion, the alignment of the cell changes. Variations can be made on the alignment of cells at the timing.

In the present study, an experimental system of the Couette type flow in the constant gap with a rotating disk has been used to apply the shear stress (2 Pa) quantitatively on the myoblast during incubation at the microscopic observation *in vitro*. The behavior of each cell has been traced at division.

2. METHODS

Shear Flow Device

To apply the continuous constant shear stress on each cell, a Couette type of shear flow device has been used in the present study: between a rotating disk and a stationary dish [19] (Fig. 1). The medium is sheared between the rotating wall and the stationary wall. The stationary wall is the bottom of the culture dish (60 mm of diameter). In the device, the shear rate (γ) in the medium is calculated by Eq. (1).

$$\gamma = r\,\omega / d \tag{1}$$

In Eq. (1), ω is the angular velocity [rad s^{-1}], and d is the distance [m] between the wall of the moving disk and the wall of stationary plate. Between the parallel walls, d is constant in the whole space. The shear rate (γ [s^{-1}]) in the gap between walls increases in proportion to the distance (r [m]) from the axis of the rotation.

The angular velocity ω (< 22 rad s^{-1}) was controlled by the stepping motor. In the observation area of the microscope, r varies between 17 mm and 18 mm. The distance d, which was measured by the positions of the focus of the walls at the microscope, was constant throughout of the gap between walls. In each test, the value d was set between 0.28 mm and 0.38 mm. Variations on the shear rates between 1×10^3 s^{-1} and 1.4×10^3 s^{-1} are made in the present experiment by adjustment of these parameters.

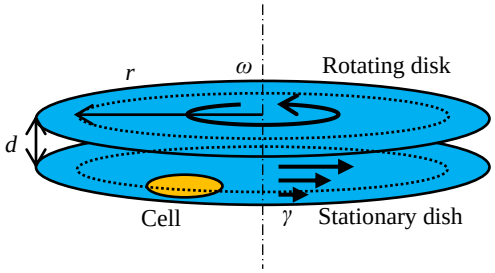

Fig. 1: Couette type of shear flow device.

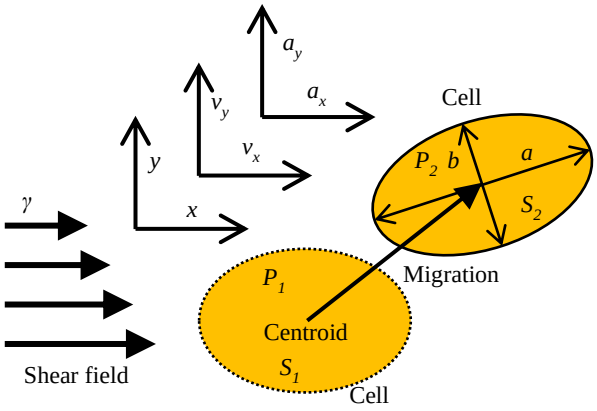

Fig. 2: Cell migration and deformation.

The shear stress (τ [Pa]) is calculated by the viscosity (η [Pa s]) of the medium (Eq. (2)).

$$\tau = \eta \gamma \tag{2}$$

Using the viscosity of the medium of 1.5×10^{-3} Pa s (measured by a cone and plate viscometer at 310 K), the variations of the shear stress τ have been calculated as the value between 1.5 Pa and 2.0 Pa (the wall shear stress).

The rotating disk device is mounted on the stage of the inverted phase contrast microscope placed in an incubator. The device allows the microscopic observation of cells cultured on the stationary wall during exposure to the shear flow.

Cell Culture

C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse, passage between eight and ten) was used in the test. Cells were cultured in the medium: Dulbecco's Modified Eagle's Medium (D-MEM)). To the medium, several contents were added: 10% of the decomplemented fetal bovine serum (FBS), sodium hydrogen carbonate ($NaHCO_3$), and 1% of penicillin/ streptomycin.

The cells were seeded on the dish with the density of 3000 cells/cm^2. To make adhesion of cells to the bottom of the culture dish, the cells were cultured for 24 hours in the incubator without flow stimulation (without rotation of the disk).

After the incubation for 24 hours, the cells were continuously sheared with the rotating disk for 7 days in the incubator at the constant rotating speed without the medium exchange. The constant speed was preset for each test to keep the designed shear stress.

Image Analysis

The time-lapse microscopic image was taken every thirty minutes during the cultivation. Cells divided during cultivation under the shear field are picked up and data are analyzed before and after 6 hours. The contour of each cell adhered on the stationary plate of the scaffold was traced, and the projected two-dimensional area (S) at the image of each cell was calculated. The rate of the area (Sr [1/hour]) was calculated by the change speed of the area from (S_1) to (S_2) per time (Δt) (Eq. (3)).

$$Sr = (S_2 - S_1) / S_1 \Delta t \tag{3}$$

The contour of each cell was approximated to ellipsoid, and the centroid of each cell was used to measure the migration (Fig. 2). The flow direction is defined as x axis. The direction to the rotational center is defined as y axis. The velocity was calculated by the change of position. The acceleration was calculated by the change of the velocity. On the ellipsoid, the length of the major axis (a), and the length of the minor axis (b) were measured. The ratio of axes is calculated as the shape index (P) by Eq. (4).

$$P = 1 - b / a \tag{4}$$

At the circle, $P = 0$. As the ellipsoid is elongated, P approaches to one. The rate of P (Pr [1/hour]) was calculated by the change speed of P (Eq. (5))

$$Pr = (P_2 - P_1) / P_1 \Delta t \tag{5}$$

3. RESULTS

Fig. 3 exemplifies the behavior of cell under the shear stress field of 1.5 Pa: before division, and after division. Fig. 4 shows behavior of cell under the shear stress field of 2.0 Pa: before division, and after division.

Figs. 3a and 4a show acceleration of the cell migration at the flow direction: under the shear stress field of 1.5 Pa and 2.0 Pa, respectively. Each cell migrates actively just after division. Figs. 3b and 4b show acceleration of the cell migration at the direction perpendicular to the flow: under the shear stress field of 1.5 Pa and 2.0 Pa, respectively. Each cell migrates to every direction. The direction is not limited to the downstream.

Figs. 3c and 4c show the shape index tracings of each cell: under the shear stress field of 1.5 Pa and 2.0 Pa, respectively. The shape index tends to increase before division. Figs. 3d and 4d show the shape index rate (Pr) of each cell: under the shear stress field of 1.5 Pa and 2.0 Pa, respectively.

Figs. 3e and 4e show the tracings of the area of each cell: under the shear stress field of 1.5 Pa and 2.0 Pa, respectively. The area of the cell tends to increase before division, especially under the higher shear field (Fig. 4e). Figs. 3f and 4f show the change rate of the area of each cell: under the shear stress field of 1.5 Pa and 2.0 Pa, respectively.

Some cells were exfoliated after decrease of the area under the shear stress field of 2.0 Pa. The term between division is shorter under the shear stress field of 1.5 Pa than 2.0 Pa. A cell with larger area was exfoliated under the shear stress field of 2.0 Pa.

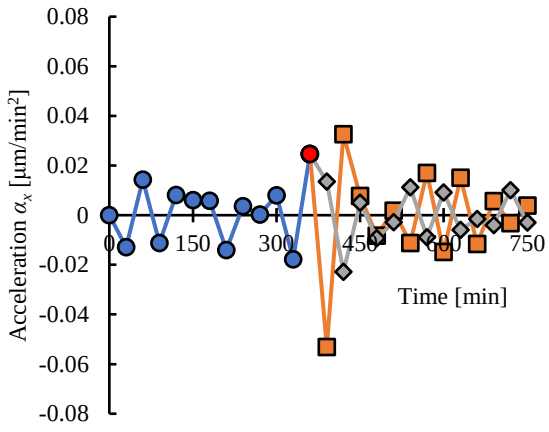

Fig. 3a: Tracings of acceleration at flow direction under shear stress of 1.5 Pa: the same marker corresponds to the same cell: cell is divided at 360 min.

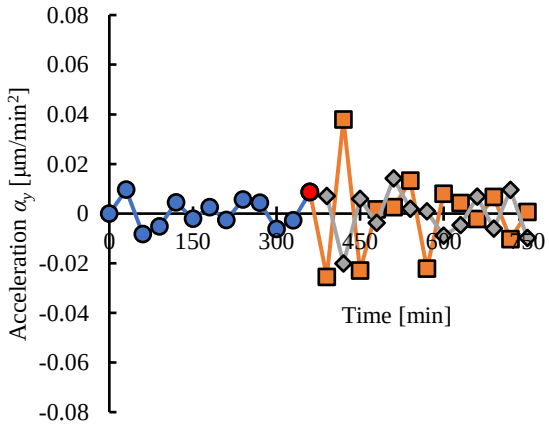

Fig. 3b: Tracings of acceleration at direction perpendicular to flow under shear stress of 1.5 Pa: the same marker corresponds to the same cell: cell is divided at 360 min.

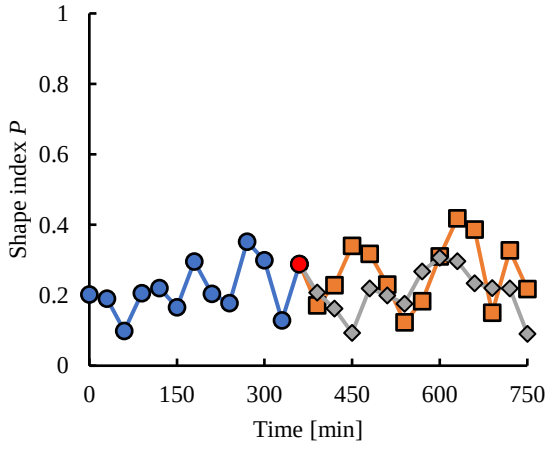

Fig. 3c: Tracings of shape index under shear stress of 1.5 Pa: the same marker corresponds to the same cell: cell is divided at 360 min.

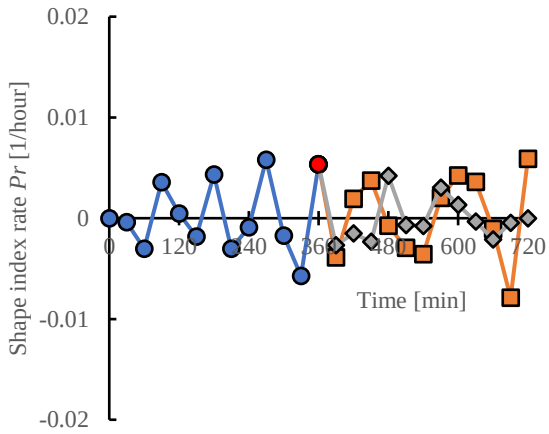

Fig. 3d: Tracings of shape index rate under shear stress of 1.5 Pa: the same marker corresponds to the same cell: cell is divided at 360 min.

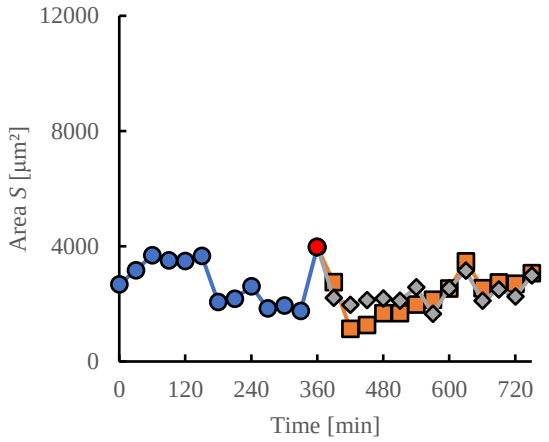

Fig. 3e: Tracings of area under shear stress of 1.5 Pa: the same marker corresponds to the same cell: cell is divided at 360 min.

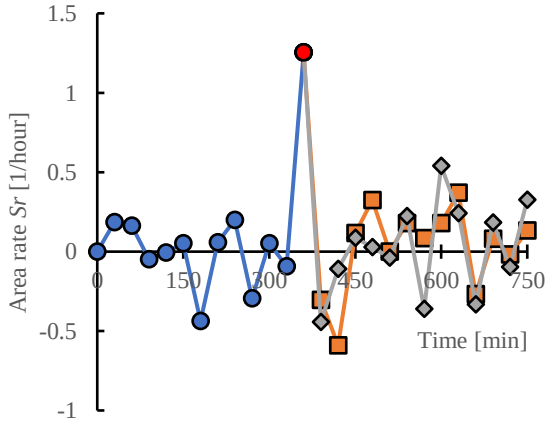

Fig. 3f: Tracings of area rate under shear stress of 1.5 Pa: the same marker corresponds to the same cell: cell is divided at 360 min.

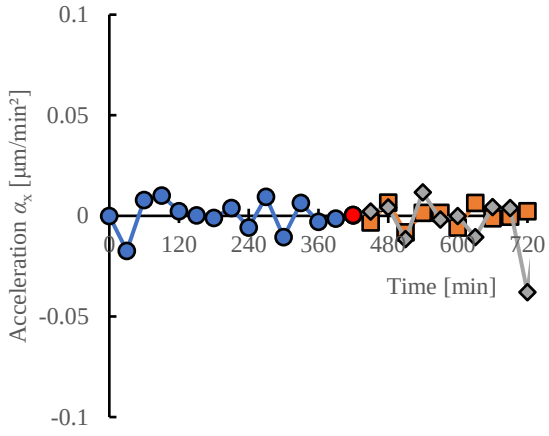

Fig. 4a: Tracings of acceleration at flow direction under shear stress of 2 Pa: the same marker corresponds to the same cell: cell is divided at 420 min.

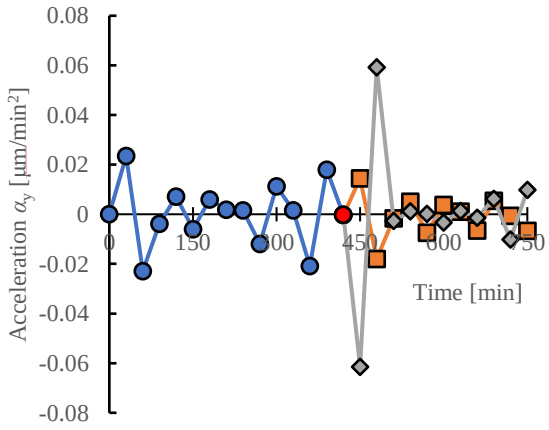

Fig. 4b: Tracings of acceleration at direction perpendicular to flow under shear stress of 2 Pa: the same marker corresponds to the same cell: cell is divided at 420 min.

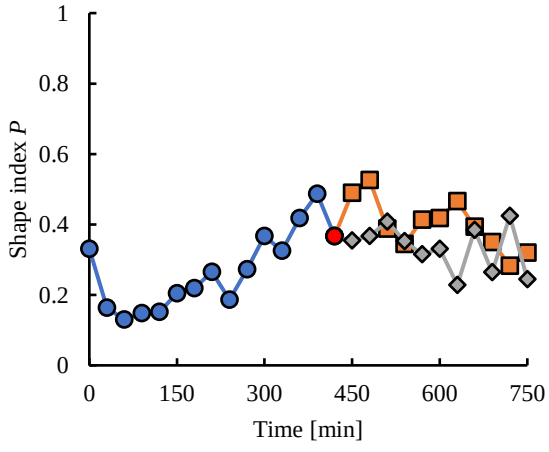

Fig. 4c: Tracings of shape index under shear stress of 2 Pa: the same marker corresponds to the same cell: cell is divided at 420 min.

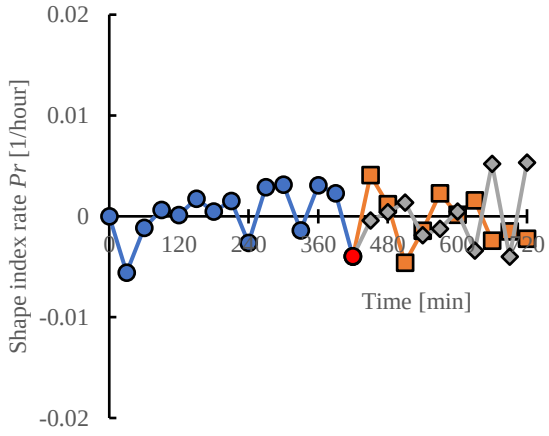

Fig. 4d: Tracings of shape index rate under shear stress of 2 Pa: the same marker corresponds to the same cell: cell is divided at 420 min.

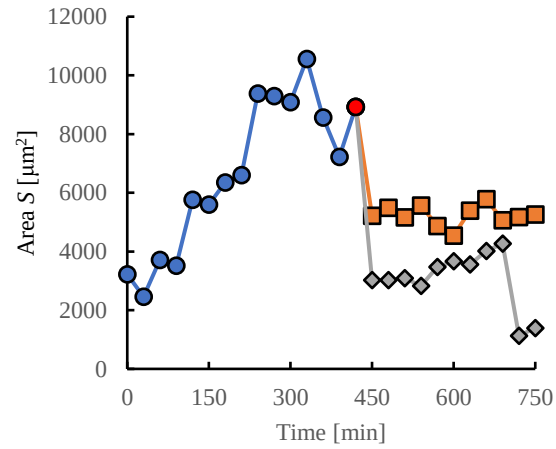

Fig. 4e: Tracings of area under shear stress of 2 Pa: the same marker corresponds to the same cell: cell is divided at 420 min.

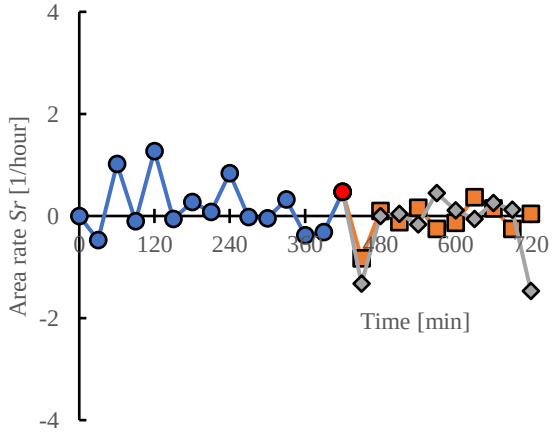

Fig. 4f: Tracings of area rate under shear stress of 2 Pa: the same marker corresponds to the same cell: cell is divided at 420 min.

4. DISCUSSION

Endothelial cells are exposed to the shear flow in the blood vessels *in vivo*. The effect of shear flow on endothelial cells was investigated in the previous studies [1-10]. Cells are exfoliated under the shear flow at the wall shear stress higher than 2 Pa [14-16]. A biological cell shows passive and active responses in an environment [6, 22]. While the flow enhances the cell migration to the downstream, a cell migrates to adapt to the shear field. While the strong stimulation above the threshold damages the cell, the stimulation below the threshold remains in the cell as a memory for the response in the next step [5, 9]. The hysteresis effect governs the active response of the cell.

In the previous study, cells were exposed to the shear flow in a donut-shaped open channel, and the effect of flow stimulation on cultured cells has been studied *in vitro* [11, 12]. When the flow has an open surface, it is difficult to estimate the shear stress value in the fluid. Between two parallel walls, on the other hand, the velocity profile is estimated to be parabolic in the laminar flow. In the previous studies, several preparations were designed to study the effect of mechanical flow stimulations on biological cells: the tilting disk channel [14], the rhombus channel [15], the cross flow channel [16], and the rotating disk type [19].

The Couette type of flow is convenient to estimate the shear stress in the flow with the constant shear rate between the moving wall and the stationary wall, which is also available to non-Newtonian fluid. Several kinds of the devices of Couette type flow were designed for quantitative experiments of biological fluid in the previous studies [19, 20]. The cone-and-plate type device has the uniform shear field in the entire space between the rotating cone and the stationary plate [20]. The shear stress is constant independent of the distance from the rotating axis.

A parallel disks system between rotating disk and the stationary disk, on the other hand, has several advantages: stability of the rotating motion of the disk, stability of the optical path for the microscopic observation, morphologic preciseness of the plane of the disks, and simultaneous observation over the range of variation of the shear rate proportional to the radius from the rotational axis.

In the present study, the rotating parallel disk system is selected to make Couette type of flow instead of the cone and plate system. At the constant angular velocity, the shear rate (γ) increases with the distance from the axis (r) in the observation area (Eq. (1)). The variation of the shear rate enables the simultaneous observation of the behavior of cells related to variation of the shear stress [19]. The rotating flow might induce the secondary flow by the centrifugal effect. The rotational speed of the disk is smaller than 0.4 m s^{-1} in the present system. The microscopic video image of the flowing cells between the rotating disk and the stationary disk shows the steady flow in the present experiment. Reynolds number (*Re*) is calculated by Eq. (6).

$$Re = \rho\, v\, d / \eta = \rho\, r\, \omega\, d / \eta \tag{6}$$

In Eq. (4), ρ is density of the fluid [kg m^{-3}], v is the circumferential velocity [m s^{-1}], ω is the angular velocity [rad^{-1}], r is the distance [m] from the rotating axis, d is the distance [m] between the moving wall and the stationary wall, and η is the viscosity of the fluid [Pa s]. *Re* is 1×10^2, when ρ, r, ω, d, and η are 1×10^3 kg m^{-3}, 0.018 m, 22 rad s^{-1}, 0.00038 m, and 0.0015 Pa s, respectively. The turbulent flow may not occur in the flow of small value of Reynolds number.

In the present experiment *in vitro*, myoblasts proliferate regardless of the shear flow stimulation (< 2 Pa). When myoblasts are cultured in the continuous steady shear flow without change of the medium between the plates, myoblasts differentiate to myotubes. The movement of each cell can be traced by the time-lapse image with the interval of thirty minutes in the present experiment.

Under the shear stress field of 2 Pa, activity of each cell decreases. Some cells are exfoliated under the wall shear stress of 2 Pa. Under the shear stress field of 1.5 Pa, on the other hand, each cell at every direction makes division (Fig. 6).

The shear stress field was also used to study adhesion phenomena in micro-biological systems [23]. In the previous study, mechanical stress was studied on differentiation of stem cells [24]. Mechanical stimulation was studied on tissue formation [25]. Property of the scaffold affects differentiation of stem cells [26, 27]. Orientation of cells can be controlled by design of the scaffold [28]. Mechanism to make orientation in the tissue might be analyzed by the study on the tracings of the alignment of each cell during division and fusion [29, 30].

5. CONCLUSIONS

The experimental results show that a cell tends to make division after the higher activity: deformation, and migration. Each cell tends to become active before and after division. A cell tends to be exfoliated after the lower activity.

ACKNOWLEDGMENT

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Effect of Shape of Cell on Movement over Micro Groove in Flow Channel

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ABSTRACT

The effect of the shape of the flowing cell on the movement over the micro groove in the micro flow channel has been investigated *in vitro*. The micro groove of the rectangular shape (4.5 μm depth) has been fabricated on the polydimethylsiloxane (PDMS) disk by the photolithography technique. The angle between the flow direction and the longitudinal axis of the groove is 45 degree. Variation has been made on the width (0.03 mm, 0.04 mm, and 0.05 mm) of the groove. A rectangular flow channel (0.05 mm height × 1 mm width × 25 mm length) has been constructed between two transparent PDMS disks. Myoblast (C2C12: mouse myoblast cell line) was used in the test. A main flow velocity (0.02 mm/s < v_x < 0.23 mm/s) of the medium was controlled by the pressure difference between the inlet and the outlet. The shape of each flowing cell was tracked on the movie recorded by the camera attached on the eyepiece of the microscope. The experimental results show that the change of the movement (perpendicular to the main flow direction) of each flowing cell at the micro oblique-groove relates to the direction change of the major axis of the cell.

Keywords: Biomedical Engineering, C2C12, Micro Groove, Micro Flow Channel and Velocity Change.

1. INTRODUCTION

The technology of sorting of cells can be applied to regenerative medicine to select the target cells [1]. It also can be applied to diagnostics to handle the target cell [2].

Several methods were proposed for the sorting of biological cells *in vitro*. The microfluidic systems were used in some methods. In these systems, variety of properties of cells were picked up for the sorting: electric property, magnetic property, dimension, density or deformability [3, 4]. In the present study, physical properties have been picked up: the shape of the floating cell.

To capture the target cell, several kinds of morphology were designed in the microfluidic systems: electrodes [5-8], micro slits [9, 10], micro holes [11, 12], or micro grooves [13-15]. The photolithography technique enables manufacturing the micro-topography [16, 17]. The movement of each cell can change at the groove on the bottom wall of the flow channel [15].

In the present study, the movement of the single cell flowing over the micro groove, which is manufactured by the photolithography technique, has been analyzed *in vitro*.

2. METHODS

Flow Channel with Micro Grooves

For changing the movement of each flowing cell, three micro grooves of the rectangular shapes (the depth of 4.5 μm depth, and 0.2 mm length) have been fabricated on the surface of the polydimethylsiloxane (PDMS) plate with the photolithography technique [15]. The grooves are arranged on the bottom of the micro flow channel. The angle between the main flow direction and the longitudinal axis of the groove is 45 degree. As to the groove arrangement from upstream to downstream, variation has been made on the width (w) of the groove: 0.03 mm, 0.04 mm, and 0.05 mm.

Both the upper and the lower plates were exposed to the oxygen gas (0.1 Pa, 30 cm^3/min) in the reactive ion etching system (FA-1) (oxygen plasma ashing, 50 W, for thirty seconds). Immediately after ashing, the upper disk adheres (plasma bonding) to the lower disk to make the flow path (0.05 mm height × 1 mm width × 25 mm length) between them. The dimension of the width of each groove was measured on the microscopic image (Fig. 2a). The flow channel is placed on the stage of the inverted phase-contrast microscope (IX71, Olympus Co., Ltd., Tokyo).

Flow Test

C2C12 (passage < 10, mouse myoblast cell line originated with cross-striated muscle of C3H mouse) was used in the test. Cells were cultured with the D-MEM (Dulbecco's Modified Eagle's Medium) containing 10% FBS and 1% of Antibiotic-Antimycotic (penicillin, streptomycin and amphotericin B, Life Technologies) in the incubator for one week.

The inner surface of the flow channel was hydrophilized by the oxygen (30 cm^3/min, 0.1 Pa) plasma ashing for one minute at 100

W by the reactive ion etching system (FA-1), and prefilled with the bovine serum albumin solution for thirty minutes at 310 K.

Before the flow test, the cells were exfoliated from the plate of the culture dish with trypsin, and suspended in the D-MEM (Dulbecco's Modified Eagle's Medium). A part (0.06 cm^3) of the suspension of cells (4000 cells/cm^3) was poured at the inlet of the flow channel. The flow (the main flow velocity (0.02 mm/s < v_x < 0.23 mm/s)) occurs by the pressure difference between the inlet and the outlet. The inlet hole (the depth of 3 mm and the diameter of 5 mm) makes the pressure head. Each cell rolling over the micro grooves was observed by the microscope, and recorded by the camera (DSC-RX100M4, Sony Corporation, Japan), which is set on the eyepiece of the microscope.

Image Analysis

"Image J" was applied to analyze the behavior of each cell. On the microscopic image, the outline of each cell was traced. The contour of each cell was approximated to ellipsoid (Fig. 1). The centroid was traced to track the movement of the cell. The coordinate was defined as follows: the direction of flow is *x*, and the direction perpendicular to the flow is *y* (Fig. 1). On the ellipsoid, the length of the major axis (*a*), and the length of the minor axis (*b*) were measured. The ratio of axes is calculated as the shape index (*P*) by Eq. (1).

$$P = 1 - b / a \tag{1}$$

At the circle, $P = 0$. As the ellipsoid is elongated, *P* approaches to one.

The angle (-90 degree < θ < 90 degree) between the direction of the main flow of the medium and the direction of the major axis of each cell was measured at the microscopic image. When the major axis is parallel to the direction of the flow, $\theta = 0$. When the major axis is perpendicular to the direction of the flow, $\theta = -90$ degree or 90 degree.

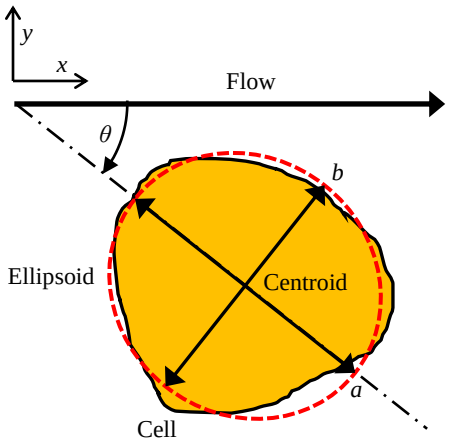

Fig. 1: Angle (θ) tracings

3. RESULTS

The tracing of the contour of each cell is exemplified in Figs. 2a, 3a, and 4a, respectively. The tracking of the shape index of each cell is exemplified in in Figs. 2b, 3b, and 4b, respectively. The tracking of the angle of the major axis of each cell is exemplified in in Figs. 2c, 3c, and 4c, respectively. The direction change of the major axis of the cell at each groove was measured as $\Delta\theta$ (Fig. 5). The tracking of the position of each cell is exemplified in in Figs. 2d, 3d, and 4d, respectively. The stepwise movement of the cell at each groove was measured as Δy (Fig. 5). Each movement of the cell over three grooves is illustrated in Figs. 2 and 3. In Figs. 2 and 3, data plot of square, rhombus, and triangle show the movement at each groove of the width: 0.03 mm, 0.04 mm, and 0.05 mm, respectively. Fig. 4 shows the movement of the cell, which does not pass over three grooves. Both the shape index and the angle of the major axis fluctuate during the movement of the cell along the medium flow. Every cell changes the direction of the movement at the groove. Both the shape index and the major axis direction also change at the groove.

Fig. 5 shows the relationship between *y* component of the stepwise movement of the cell (Δy) and the direction change of the major axis of the cell ($\Delta\theta$). Figs. 5a, 5b, and 5c show data at each groove of the width: 0.03 mm, 0.04 mm, and 0.05 mm, respectively. The dotted line is the regression line of data in each figure (Figs. 5a, 5b, 5c). The correlation coefficient of data is calculated as *r* value in the figure. The stepwise movement (Δy) increases with the increase of the angle change ($\Delta\theta$) at the groove. The tendency is remarkable at the wider groove.

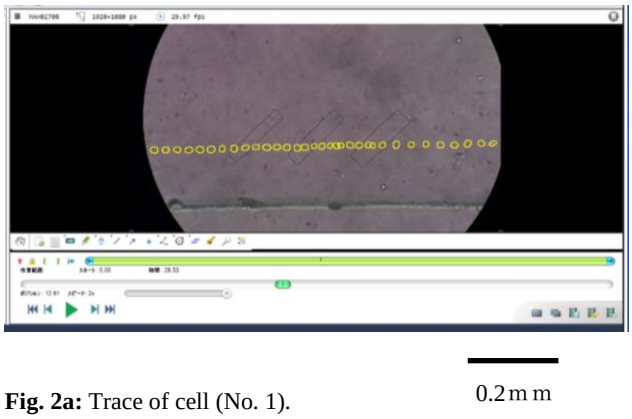

Fig. 2a: Trace of cell (No. 1).

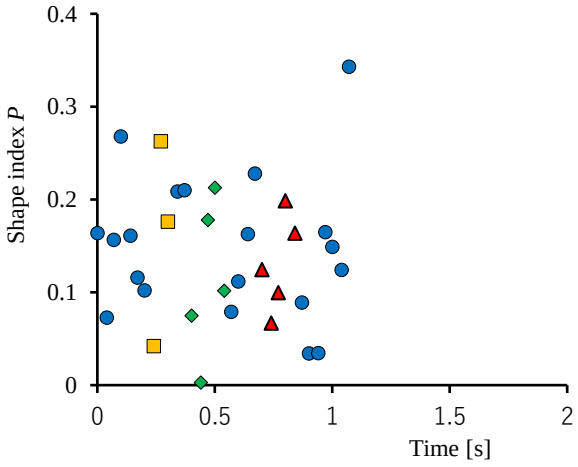

Fig. 2b: Shape index (*P*) tracings: over groove of *w* = 0.03 mm (square: 0.263-0.042), 0.04 mm (rhombus: 0.213-0.003), 0.05 mm (triangle: 0.199-0.067): (No. 1).

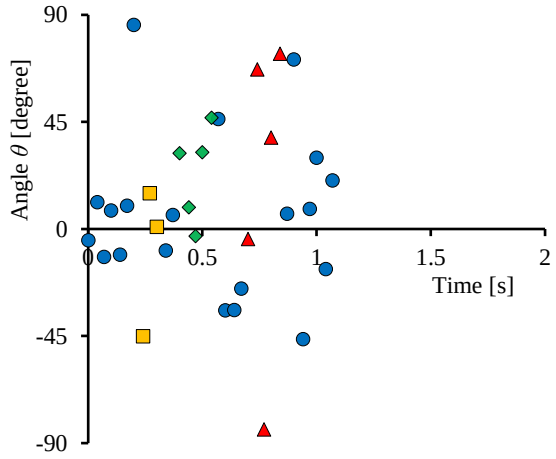

Fig. 2c: Angle (θ) tracings: over groove of w = 0.03 mm (square: 15.0-(-45.1)), 0.04 mm (rhombus: 46.8-(-3.02)), 0.05 mm (triangle: 73.7-(-84.3)) (No. 1).

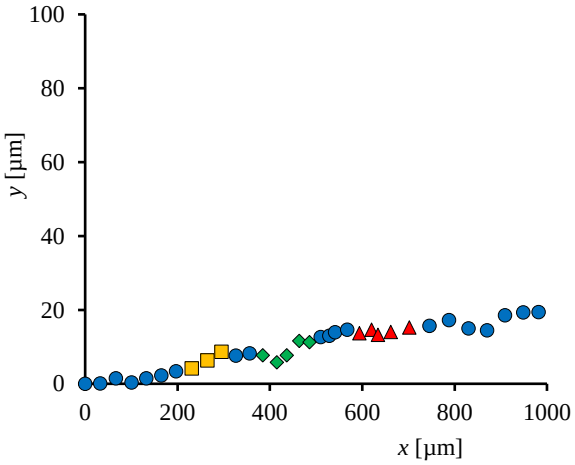

Fig. 2d: Movement of cell: 266.4 μm^2: over groove of w = 0.03 mm (square), 0.04 mm (rhombus), 0.05 mm (triangle) (No. 1).

Fig. 3a: Trace of movement of cell (No. 2).

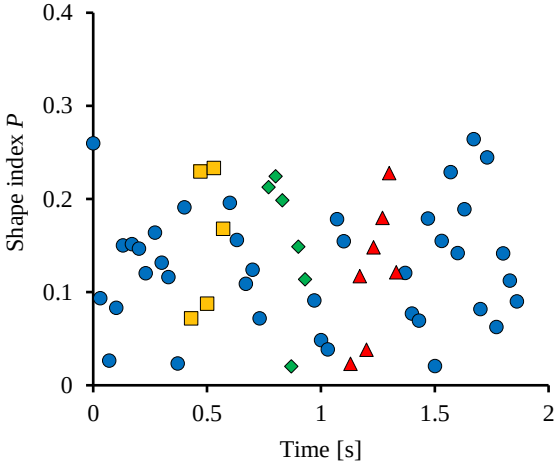

Fig. 3b: Shape index tracings: over groove of w = 0.03 mm (square), 0.04 mm (rhombus), 0.05 mm (triangle): (No. 2).

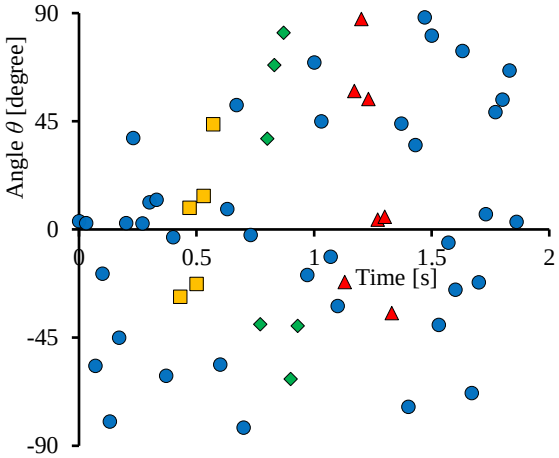

Fig. 3c: Angle (θ) tracings: over groove of w = 0.03 mm (square), 0.04 mm (rhombus), 0.05 mm (triangle): (No. 2).

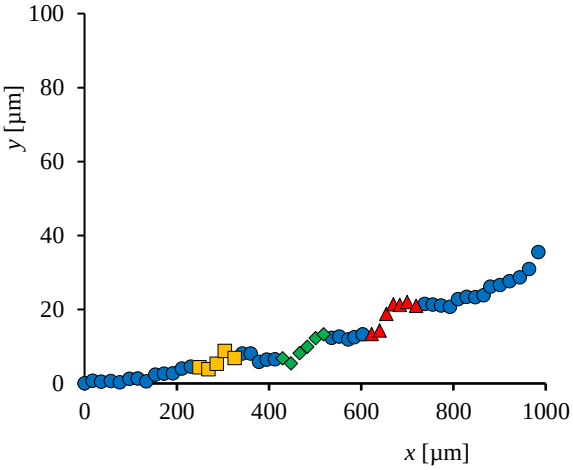

Fig. 3d: Movement of cell: over groove of w = 0.03 mm (square), 0.04 mm (rhombus), 0.05 mm (triangle) (No. 2).

Fig. 4a: Trace of movement of cell: control (No. 3).

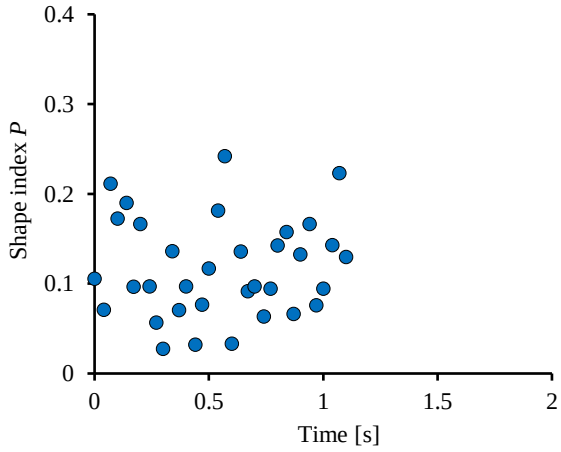

Fig. 4b: Shape index tracings: control (No. 3).

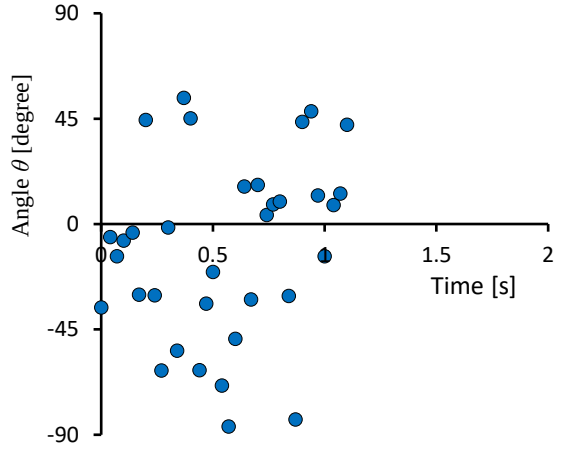

Fig. 4c: Angle (θ) tracings: control (No. 3).

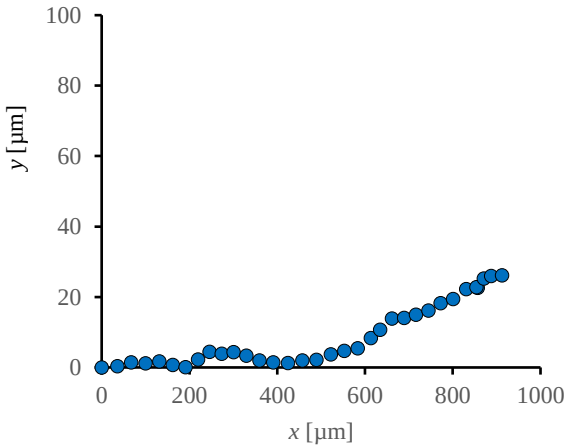

Fig. 4d: Movement of cell: control (No. 3).

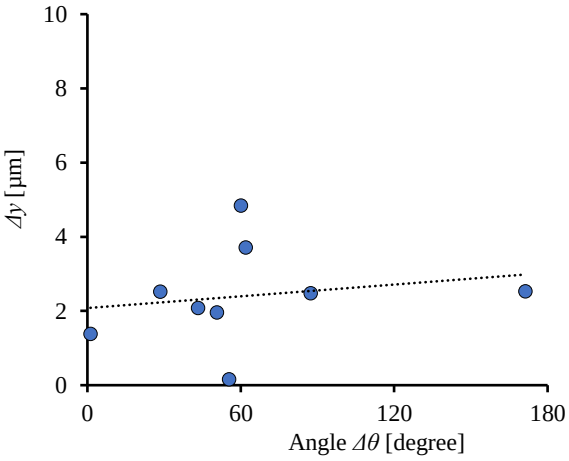

Fig. 5a: Relationship between step (Δy) and angle ($\Delta\theta$) over groove of 30 μm: dotted line, $\Delta y = 0.0053\,\Delta\theta + 2.1$, $r = 0.19$.

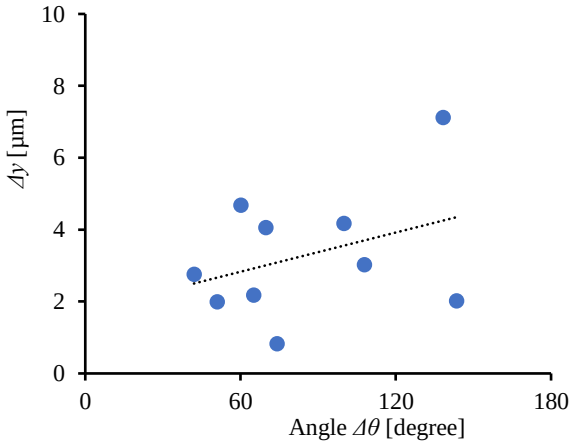

Fig. 5b: Relationship between step (Δy) and angle ($\Delta\theta$) over groove of 40 μm: dotted line, $\Delta y = 0.018\,\Delta\theta + 1.7$, $r = 0.36$.

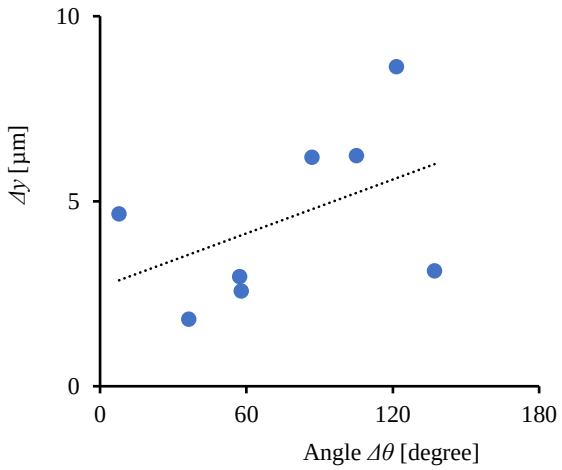

Fig. 5c: Relationship between step (Δy) and angle ($\Delta\theta$) over groove of 50 μm: dotted line, $\Delta y = 0.024\,\Delta\theta + 2.7$, $r = 0.46$.

4. DISCUSSION

The shape of the cell can be detected by the movement of the flowing cell over the micro groove. The position of y coordinate gradually increases, because the direction of the main flow tilts (0.02 mm / 1 mm) from horizontal direction in Fig. 4d.

Filtration is one of the basic methods of sorting [18]. Fluorescence techniques [19-21] was used in the previous sorting systems with flow cytometry technique [22]. Non-destructive cell sorting systems, on the other hand, were designed in the previous studies [23]. The label-free methods were designed with microfluidic systems [24, 25]. Some of them were designed to capture cancer cells [26].

The microfluidic system was applied to sort biological cells [27-31], and to trap biological cells [32, 33]. The sorting technique might be applied to selection of cells for regenerative medicine and diagnostics of disease.

Several kinds of systems were designed for the cell sorting in vitro [9, 20, 21]. The micromachining technique has been applied to cell technology [34].

Several fluid flow systems were used in the previous studies. In the previous studies, cylindrical [11] and half cylindrical [12] holes were used for the trap of cells. The asymmetrical hole [12] might be suitable for trap than the symmetrical hole.

The rectangular grooves have been successfully manufactured on the wall of the micro fluid channel. The dimension of the grooves was confirmed by the laser microscope [13].

The depths of the micro patterns were between 2 μm and 10 μm in the previous studies [19-22]. In the present study, the depth of the grooves is (4.5 μm, which is smaller than the diameter of the cells. The deeper hole may have advantage to trap every cell. At the shallower trap, on the other hand, it is not easy to tarp a cell. The trap of the appropriate dimension can distinguish cells. The duration of the trapped time of the cell might relate to interaction between the micro hole and the cell: affinity between the cell and the surface of the micro pattern, or deformability of the cell.

The results of the previous study show that the movement of cell travelling on the wall is modified by the oblique micro groove on the wall under the cell velocity lower than 1 mm/s [21]. The angle of 45 degrees between the longitudinal direction of the groove and the flow direction is effective to shift the streamline of the cell. The shift movement along the oblique groove depends on the several parameters: the diameter of cells, the width of the groove, the velocity of the cell, and the cell types [13]. As the diameter of the cell decreases, the traveling length along the groove increases. The movement may be related not only to the diameter but also to deformability of the cell. Deformability was measured on red blood cells in previous studies [35].

The movement of flowing cell at the bottom surface of the flow channel may be related to the specific gravity of the cell: the density difference between the cell and the medium. The shifted distance of malnourished cells by the oblique groove was smaller than that of normal cells in the previous study [15].

In the present study, cells are sparsely suspended in the medium flow to reduce the interaction between cells. Cells can be sorted by the velocity change at the micro groove according to the diameter and the width of the groove [36].

5. CONCLUSIONS

The change of the movement (perpendicular to the main flow direction) of each flowing cell at the oblique groove relates to the direction change of the major axis of the cell.

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Effect of Contact between Myoblasts on Making Orientation of Cells under Shear Flow Field

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ABSTRACT

The effect of contact between cells on making orientation of cells under the shear flow field has been investigated *in vitro*. To make a Couette type of the shear flow, the culture medium was sandwiched with a constant gap between parallel walls: a lower stationary culture disk, and an upper rotating disk. The wall shear stress (2 Pa) on the lower culture disk was controlled by the rotating speed of the upper disk. Myoblasts (C2C12: mouse myoblast cell line) were used in the test. After cultivation without flow for 24 hours for adhesion of cells on the lower plate, the constant wall shear stress was continuously applied on cells for 7 days in the incubator. The behavior of each cell was traced at the time-lapse images observed by an inverted phase contrast microscope placed in an incubator. The experimental results quantitatively show that increase of the contact region between cells affects making perpendicular orientation of cells against the main flow direction.

Keywords: Biomedical Engineering, Interaction between Cells, Wall Shear Stress, C2C12 and Orientation.

1. INTRODUCTION

Biological cells make orientation in the tissue. The orientation relates to the function of the tissue. The process to make orientation of cells in the tissue depends on the surrounding environment [1]. The mechanical force field is one of the environments [2]. The effects of the shear flow on the endothelial cells, which are exposed to the blood flow on the inner surface of the vessel wall [3], were investigated in many studies [4]. Endothelial cells make orientation on the inner surface of the blood vessel wall. The orientation is parallel to the blood flow direction. In the previous study with the vortex flow by the swinging plate in vitro, C2C12 (mouse myoblast cell line) made orientation perpendicular to the direction of the flow, although HUVEC (human umbilical vein endothelial cell) made orientation along the streamline of the flow [5].

The alignment of each cell also depends on that of the neighbor cell. The alignment of the cell tends to follow that of neighbor cell to make parallel orientation each other. In the present study, the effect of interaction between cells on making orientation of cells under the shear flow field has been investigated *in vitro*.

2. METHODS

Shear Flow Device
A Couette type of shear flow device has been used in the present study: between a rotating disk and a stationary dish (Fig. 1). The medium is sheared between a rotating wall and a stationary wall. The stationary wall is the bottom of the culture dish (diameter 60 mm).

In the devise, the shear rate (γ) in the medium is calculated by Eq. (1).

$$\gamma = r\,\omega / d \tag{1}$$

In Eq. (1), ω is the angular velocity [rad s^{-1}], and d is the distance [m] between the wall of the moving disk and the wall of stationary plate. Between the parallel walls, d is constant.

The angular velocity ω (22 rad s^{-1}) was controlled by the stepping motor. In the observation area of the microscope, r varies between 17 mm and 18 mm. The distance d, which was measured by the positions of the focus of the walls at the microscope, was 0.29 mm. The shear rate (γ) is set at 1.3×10^{3} s^{-1} in the present experiment by adjustment of these parameters.

The shear stress (τ [Pa]) is calculated by Eq. (2).

$$\tau = \eta\,\gamma \tag{2}$$

In Eq. (2), η is the viscosity [Pa s] of the medium. Using the viscosity of the medium of 1.5×10^{-3} Pa s (measured by a cone and plate viscometer at 310 K), the shear stress τ is calculated as 2.0 Pa. The rotating disk device is mounted on the stage of the inverted phase contrast microscope placed in the incubator. The

device allows the microscopic observation of cells cultured on the stationary wall during exposure to the shear flow.

Cell Culture

C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse, passage between eight and ten) was used in the test. Cells were cultured in the Dulbecco's Modified Eagle's medium (D-MEM): containing 10% of the decomplemented fetal bovine serum (FBS), sodium hydrogen carbonate ($NaHCO_3$), and 1% of penicillin/ streptomycin.

The cells were seeded on the dish at the density of 3000 cells/cm^2. To make adhesion of cells to the bottom of the culture dish, the cells were cultured for 24 hours in the incubator without flow stimulation (without rotation of the disk).

After the incubation for 24 hours, the cells were continuously sheared with the rotating disk for 7 days in the incubator at the constant rotating speed without the medium exchange. The constant speed was preset for each test to keep the designed shear stress.

Image Analysis

The time-lapse microscopic images were taken every thirty minutes during the cultivation. The contour of each cell adhered on the stationary plate of the scaffold was traced (Fig. 2), and was approximated to the ellipsoid.

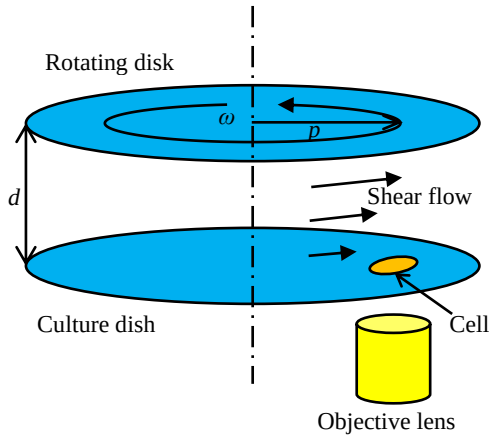

Fig. 1: Shear flow between rotating disk and stationary dish.

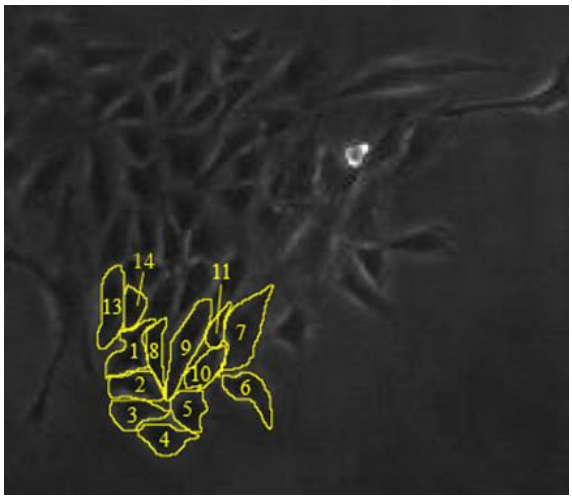

Fig. 2a: Traced single cells (1-14) in area A.

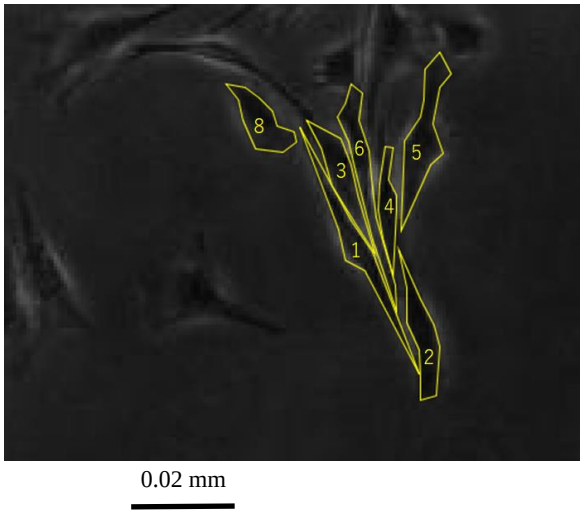

Fig. 2b: Traced single cells (1-8) in area B.

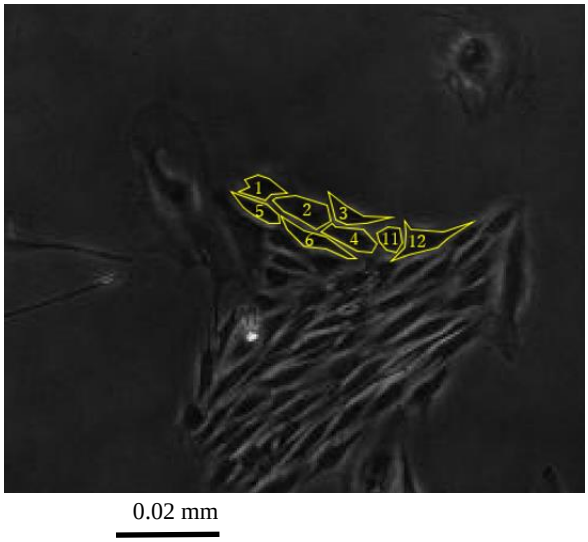

Fig. 2c: Traced single cells (1-12) in area C.

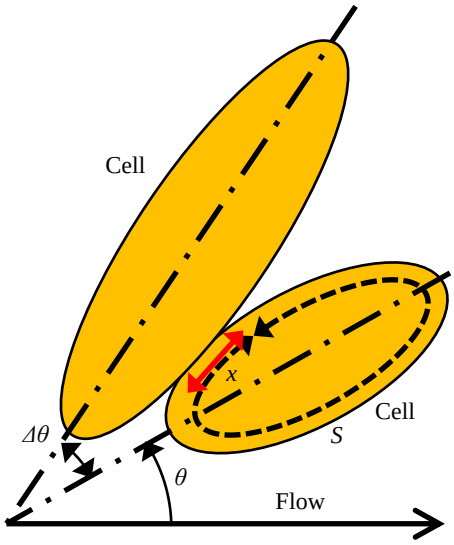

Fig. 3: Contact between cells.

The angle (0 degree < θ < 180 degree) between the longitudinal axis of the cell and the flow direction was measured at the microscopic image of each cell. The alignment of each cell was traced for 24 hours under the continuous shear flow stimulation.

The length of the contact line (x) between cells was measured on the image (Fig. 3). The length ratio R is calculated by Eq. (3).

$$R = x / S \quad (3)$$

S is the peripheral length of the smaller cell.

3. RESULTS

Myoblasts showed every activity during cultivation under the continuous constant wall shear field: migration, exfoliation, deformation, division, and fusion to make myotubes. Fig. 4 exemplifies tracings of the angle (θ) between the longitudinal axis of the cell and the flow direction for 24 hours in the colony in the area B. Every cell in Fig. 4 approaches to 90 degrees, which indicates making orientation perpendicular to the flow direction. The length of the contact line (x) between cells varies during the period (Fig. 5). In the following figures, the contact between cells is evaluated by the length ratio.

Fig. 6 shows the angle between cells ($\Delta\theta$) in relation to the length ratio (R) in the colony in the area A (Fig. 2a). Fig. 7 shows the angle between cells ($\Delta\theta$) in relation to the length ratio (R) in the colony in the area B (Fig. 2b). Fig. 8 shows the angle between cells ($\Delta\theta$) in relation to the length ratio (R) in the colony in the area C (Fig. 2c). The regression lines are added to data between cells, which has wider variation of the length ratio (R). The correlation coefficient (r) was calculated for each regression line.

Regression lines in Figs. 6a-6e have negative slopes. The correlation coefficients (r) are in the range between 0.31 and 0.62 in Figs. 6a-6d. When the angle between cells ($\Delta\theta$) is larger than 30 degrees, the angle $\Delta\theta$ tends to decrease with the length ratio (R). When the angle $\Delta\theta$ is smaller than 30 degrees, on the other hand, the angle does not decrease every time (Fig. 7). In some cases, the angle $\Delta\theta$ increases with the length ratio (R) (Fig. 7g, Fig. 7b, Figs. 8a, 8b).

Fig. 7 shows the angle ($\Delta\theta$) in relation to the length ratio (R), when the longitudinal direction of each cell is perpendicular to the wall shear stress direction. Slopes between $\Delta\theta$ and R are very small. Angles ($\Delta\theta$) are maintained smaller than 30 degrees. The parallel position between cells is kept in the direction perpendicular to the flow.

Fig. 8 shows the angle ($\Delta\theta$) in relation to the length ratio (R), when the longitudinal direction of each cell is parallel to the wall shear stress direction. When the angle between cells ($\Delta\theta$) is larger than 30 degrees, the angle $\Delta\theta$ tends to decrease with the length ratio (R) (Fig. 8a, Fig. 8b). Although angles ($\Delta\theta$) smaller than 30 degrees are maintained, the angle $\Delta\theta$ increases with the length ratio (R) (Fig. 8b) in some cases.

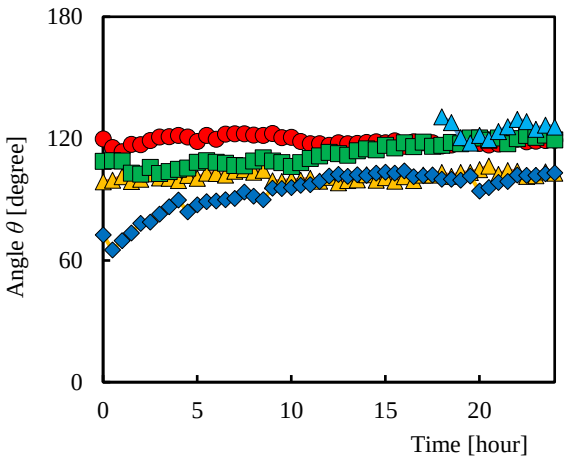

Fig. 4: Tracings of angle θ of cell.

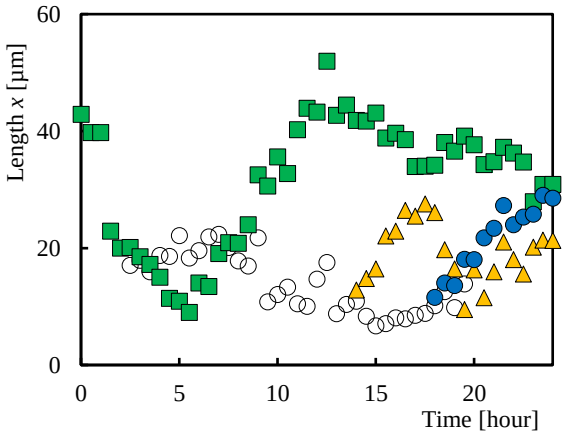

Fig. 5: Tracings of contact length x between cells.

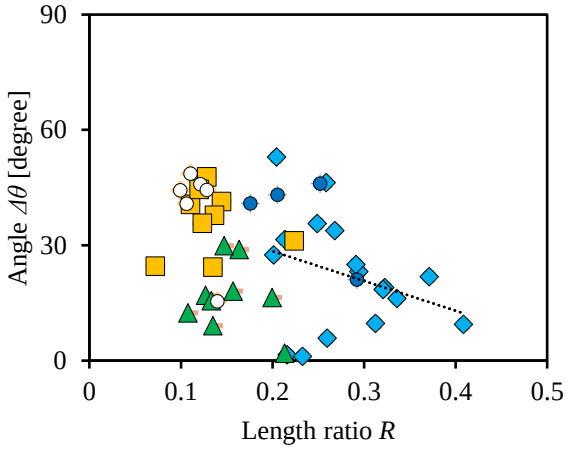

Fig. 6a: Length ratio R vs. angle $\Delta\theta$ in contact of cell 1 with cell 14 (open circle), cell 8 (blue circle), cell 13 (square), cell 12 (triangle), and cell 2 (rhombus): dotted line, regression line between cell 1 and 2 ($\Delta\theta = -77\ R + 44$): correlation coefficient r = 0.31.

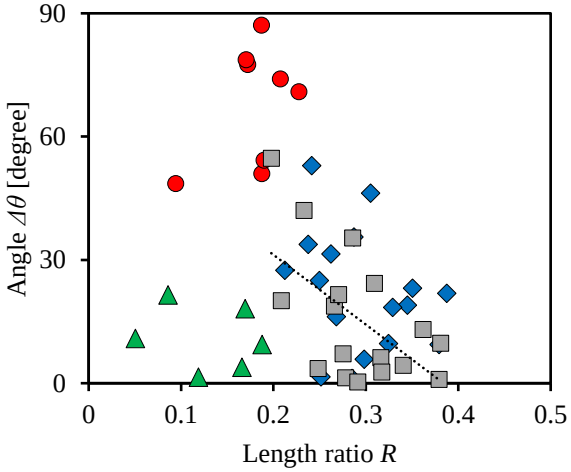

Fig. 6b: Length ratio R vs. angle $\Delta\theta$ in contact of cell 2 with cell 8 (circle), cell 3 (square), cell 12 (triangle), and cell 1 (rhombus): dotted line, regression line between regression line between cell 2 AND 3 ($\Delta\theta$ = 170 R + 65): correlation coefficient r = 0.58.

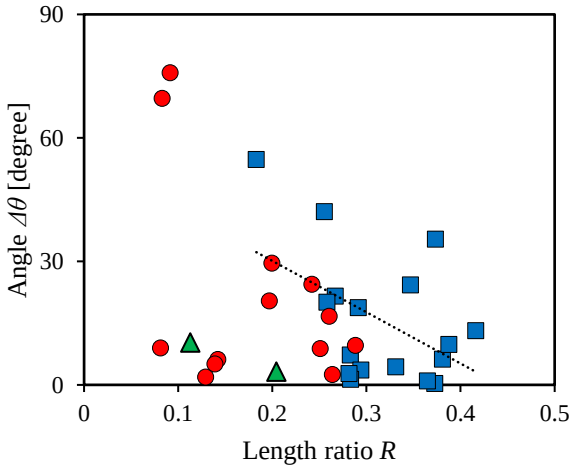

Fig. 6c: Length ratio R vs. angle $\Delta\theta$ in contact of cell 3 with cell 8 (circle), cell 2 (square), and cell 4 (triangle): dotted line, regression line between cell 2 and 3 ($\Delta\theta$ = −125 R + 55): correlation coefficient r = 0.48.

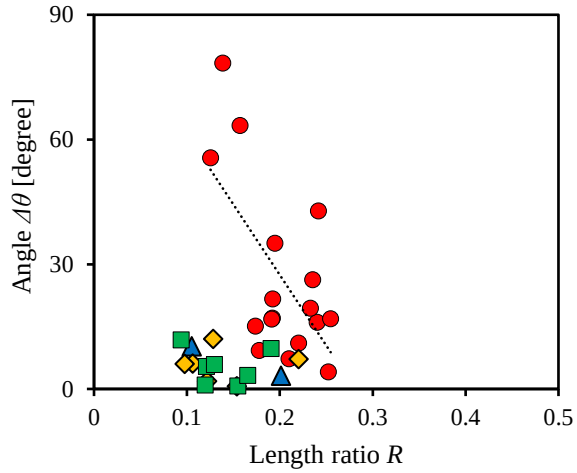

Fig. 6d: Length ratio R vs. angle $\Delta\theta$ in contact of cell 4 with cell 5 (circle), cell 9 (square), cell 3 (triangle), and cell 8 (rhombus): dotted line, regression line between cell 4 and 5 ($\Delta\theta$ = −339 R + 95): correlation coefficient r = 0.62.

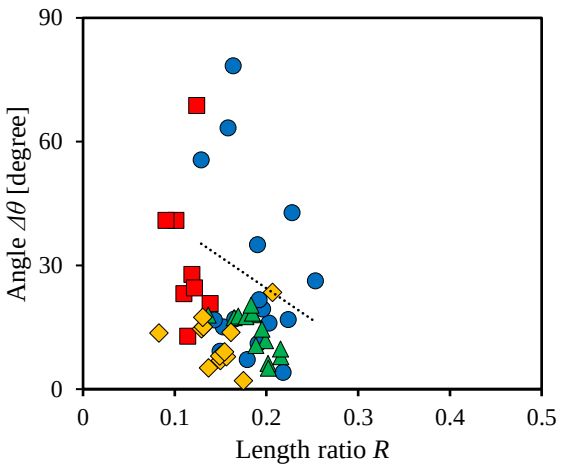

Fig. 6e: Length ratio R vs. angle $\Delta\theta$ in contact of cell 5 with cell 4 (circle), cell 6 (square), cell 9 (triangle), and cell 10 (rhombus): dotted line, regression line between cell 4 and 5 ($\Delta\theta$ = −152 R + 55): correlation coefficient r = 0.24.

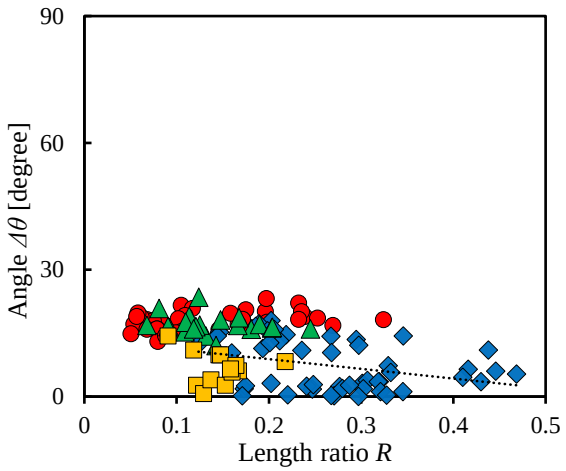

Fig. 7a: Length ratio R vs. angle $\Delta\theta$ in contact of cell 1 with cell 2 (circle), cell 8 (square), cell 6 (triangle), and cell 3 (rhombus): dotted line, regression line between cell 1 and 3 ($\Delta\theta$ = − 23 R + 13): correlation coefficient r = 0.33.

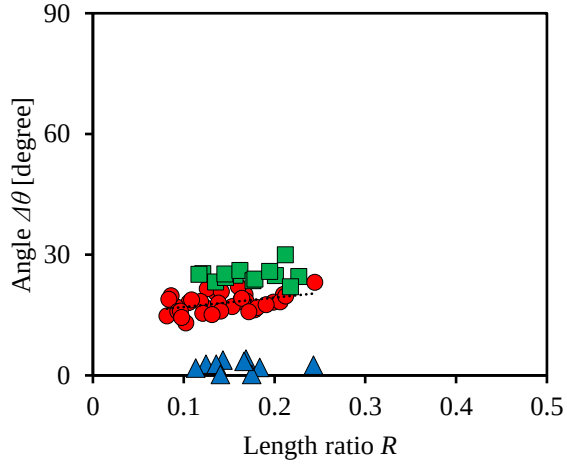

Fig. 7b: Length ratio R vs. angle $\Delta\theta$ in contact of cell 2 with cell 1 (circle), cell 5 (square), and cell 6 (triangle): dotted line, regression line between cell 1 and 2 ($\Delta\theta$ = 23 R + 15): correlation coefficient r = 0.44.

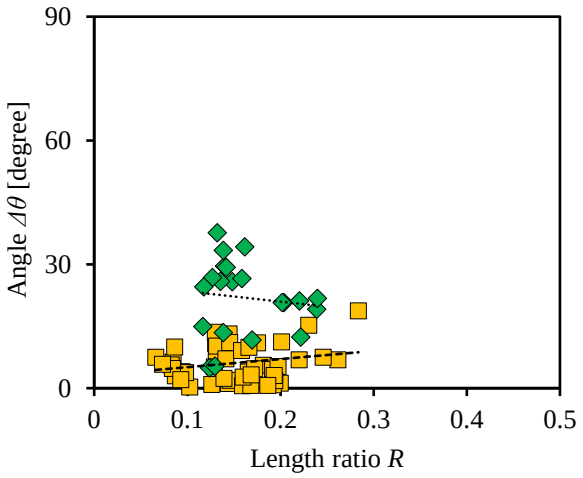

Fig. 8a: Length ratio R vs. angle $\Delta\theta$ in contact of cell 8 with cell 1 with cell 2 (square), and cell 5 (rhombus): dotted line, regression line between cell 1 and 5 ($\Delta\theta = -25\ R + 26$): correlation coefficient $r = 0.11$: broken line, regression line between cell 1 and 2 ($\Delta\theta = 20\ R + 3$): correlation coefficient $r = 0.23$.

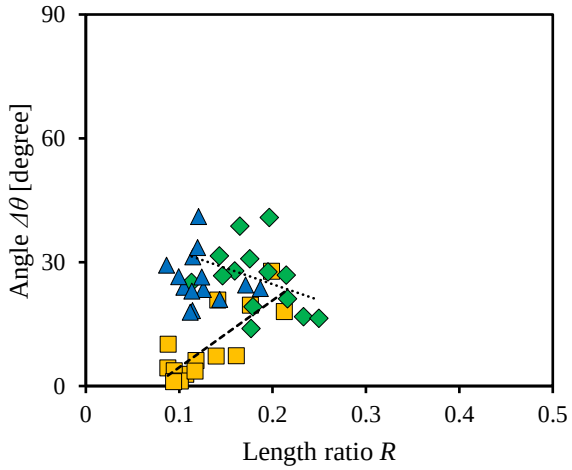

Fig. 8b: Length ratio R vs. angle $\Delta\theta$ in contact of cell 8 with cell 7 (square), cell 12 (triangle), and cell 10 (rhombus): dotted line, regression line between cell 8 and 10 ($\Delta\theta = -77\ R + 40$: correlation coefficient $r = 0.36$: broken line, regression line between cell 7 and 8 ($\Delta\theta = 162\ R - 12$): correlation coefficient $r = 0.80$.

4. DISCUSSION

To study on the orientation of cells, the orientation should be evaluated quantitatively by the parameter. The shape of each C2C12 adhered on the culture plate can be approximated to the ellipsoid. In the previous study, the distribution of the direction of the longitudinal axis of each ellipsoid has been measured [6]. Under the wall shear stress, a cell shows the following responses: elongation, tilting to the streamline, migration, deformation to be rounded, division, and exfoliation from the wall of the scaffold. In the Poiseuille type of flow, the shear rate depends on the distance from the wall: zero at the center of the flow-path, and the highest value at the wall. In the Couette type of flow, on the other hand, the shear rate is constant regardless of the distance from the wall [7].

The experimental results show that each single cell tends to align parallel to the flow direction under shear stress of 2 Pa in the previous study [6]. The mean direction of cells in some colonies of C2C12 tends to align perpendicular to the flow direction. Because of the interaction of cells, the direction of the cell depends on that of the neighbor cell. Myotubes also make fusion to make myotubes. The results would be applied to make orientation of myotubes in an engineered tissue. The experimental methods to control the shear field of the medium in the flow would be applied to the acceleration technique to make orientation of cells *in vitro*.

When the angle between cells ($\Delta\theta$) is larger than 30 degrees, the contact between cells affect the angle change. The smaller cell tends to align parallel to the neighbor cell [8]. When the angle between cells is small, the parallel position between cells is maintained. In these processes, the orientation of cells perpendicular to the flow direction can be made in the colony.

The interaction between cells governs the behavior of each cell. The orientation of each cell depends on the orientation of the neighbor cell. Myoblasts tend to migrate to the oblique direction under the shear stress field of 1.5 Pa [9]. The effect of shear flow on cells depends on the cell types. The dependency might be applied to the cell sorting technology [10, 11]. The quantitative relationships between the shear stress and the cell orientation might be applied to tissue technology to control of cells *in vitro*.

In the tissue technology, orientation of cells was controlled by the design of the scaffold *in vitro*. Alignment of fibers of the scaffold was controlled by the electrospinning technique [12]. 3D-printing technology was applied to make orientation of cells [13]. The Micro-robotic technique was applied to the cell manipulation to control orientation of each cell [14].

Endothelial cells are exposed to the shear flow in the blood vessels *in vivo*. The shear flow affects vessel wall [15, 16] and clot formation [17]. The effects of shear flow on endothelial cells were investigated in the previous studies [18, 19,20]. Cells are exfoliated under the shear flow at the wall shear stress higher than 2 Pa [21-23]. A biological cell shows passive and active responses in an environment [18]. While the flow enhances the cell migration to the downstream, a cell migrates to adapt to the shear field. While the strong stimulation above the threshold damages the cell, the stimulation below the threshold remains in the cell as a memory for the response in the next step [19, 20]. The hysteresis effect governs the active response of the cell.

5. CONCLUSIONS

The experimental results quantitatively show that increase of the contact region between cells affects making perpendicular orientation of cells against the main flow direction.

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Tracking of Deformation of Cell during Passing through Micro Gap

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ABSTRACT

Deformation of each cell during passing through a micro-slit in a flow channel has been investigated *in vitro*. At the middle part of the flow channel, the slit with the rectangular cross section (7 μm height, 0.4 mm width, and 0.1 mm length) has been made by the photolithography technique. Myoblasts (C2C12: mouse myoblast cells) were used in the test. The flow rate of the medium, in which cells were suspended, was controlled by the pressure head between the inlet and the outlet. The deformation of cells passing through the micro slit was observed with an inverted phase-contrast microscope. The experimental results show intermittent deformation of each cell quantitatively through the gap.

Keywords: Biomedical Engineering, C2C12, Deformation, Photolithography and Micro-slit.

1. INTRODUCTION

The deformation of the biological cell relates to the biological function. An erythrocyte has a high flexibility to pass through the capillary in the micro circulation system for the blood *in vivo*. Several kinds of the flowing cells can pass through the narrow slit *in vivo*.

The deformability of the biological cell plays an important role in vivo. A red blood cell, for example, has high flexibility. The elasticity of the membrane [1], and fatigue [2] were evaluated in the previous studies. The aging effect on deformability [3] and the sublethal damage [4] were also detected in the shear flow in the previous studies. It also passes through micro-circulation, of which the dimension is smaller than the diameter of the red blood cell. After circulation through the blood vessels for days, the red blood cell is trapped in the micro-circulation systems.

Several systems sort cells according to the deformability in vivo. One of the systems, which trap red blood cells, is a spleen. The spleen has special morphology at the blood flow path to sort injured red blood cells [5]. A slit is one of the systems, which sorts biological cells in vivo. The sorting at the slit depends on the deformability of each cell. Some cells are able to pass through a very narrow slit.

A photolithography technique enables manufacturing micro grooves [6] or micro structures [7] in the flow-channel for cell tests in vitro. Several micro-fabrication processes have been designed to simulate morphology of the microcirculation [8]. The technique also has been applied to handle cells in diagnostics in vitro [9]. The photolithography technique can be applied to make a micro slit. The slit between micro cylinders was made to sort cells in the previous study [10]. The deformation of the depth direction between cylinders, however, cannot be observed by the conventional optical microscope. To observe the deformed cell at the direction perpendicular to the walls of the slit, another type of the slit is designed with the combination of micro ridges in the previous study [11].

In the present study, deformation of each cell during passing through a micro-slit in a flow channel has quantitatively been investigated *in vitro*.

2. METHODS

Micro Slit

A micro slit has been designed between a transparent polydimethylsiloxane (PDMS) plate and a borosilicate glass (Tempax) plate. The upper plate of PDMS has a rectangular ridge of 0.05 mm height, 0.10 mm width, and 2 mm length. The lower plate of glass has a rectangular groove (the depth of 0.010 mm, the width of 2 mm, and the length of 20 mm), which has a narrow part of 0.8 mm width. These plates make contact to keep slits (the width of 0.8 mm, the length of 0.1 mm and the height of 0.010 mm) between them.

Flow Channel

The upper plate of PDMS was rinsed with IPA, and with the ultrapure water. The plate was dried by the spin-dryer. The surface of the PDMS plate was hydrophilized by the oxygen (30 cm^3/min, 0.1 Pa) plasma ashing for thirty seconds at 50 W by the reactive ion etching system (FA-1). The plate was rinsed with APTES (Aminopropyl-triethoxysilane) for five minutes, and with the ultrapure water. The plate was dried in the oven at 338

K for three minutes. The upper plate of PDMS was adhered on the lower plate of SU8-10 to make the slit, and was baked on the heated plate at 338 K for five minutes.

Flow Test

C2C12 (passage < 10, mouse myoblast cell line originated with cross-striated muscle of C3H mouse) was used in the test. Cells were cultured with the D-MEM (Dulbecco's Modified Eagle's Medium) containing 10% FBS and 1% of Antibiotic-Antimycotic (penicillin, streptomycin and amphotericin B, Life Technologies) in the incubator for one week.

The inner surface of the flow channel was hydrophilized by the oxygen (30 cm^3/min, 0.1 Pa) plasma ashing for one minute at 100 W by the reactive ion etching system (FA-1), and prefilled with the bovine serum albumin solution for thirty minutes at 310 K.

Before the flow test, the cells were exfoliated from the plate of the culture dish with trypsin, and suspended in the D-MEM (Dulbecco's Modified Eagle's Medium). The suspension of cells (4000 cells/cm^3, 0.06 cm^3) was poured at the inlet of the flow channel. The flow occurs by the pressure difference between the inlet and the outlet. The inlet hole (the depth of 3 mm and the diameter of 5 mm) makes the pressure head. The behavior of cells near the slit was observed with an inverted phase-contrast microscope (IX71, Olympus Co., Ltd., Tokyo) at 298 K.

The microscopic movie images of thirty frames per second at the shutter speed of 1/2000 s were recorded by the camera (DSC-RX100M4, Sony Corporation, Tokyo, Japan) (Fig. 1).

The velocity of the cell passing through the slit (v) was calculated at the movie images using "Kinovea". Several cells pass through the slit in a few seconds. Data of clogging cell in the slit are not included in the following figures.

At the image, the contour of each cell was traced, and analyzed by "ImageJ". The projected two-dimensional area (S) was calculated.

The contour was approximated to the ellipsoid. On the ellipsoid, the length of the major axis (a), and the length of the minor axis (b) were measured (Fig. 2). The ratio of axes is calculated as the shape index (P) by Eq. (2).

$$P = 1 - b / a \tag{2}$$

At the circle, $P = 0$. As the ellipsoid is elongated, P approaches to one.

The angle θ (-90 degree $< \theta <$ 90 degree) between the major axis and the medium flow direction was measured (Fig. 2).

3. RESULTS

Figs. 3 and 4 exemplify the behavior of each cell in the slit. Figs. 3a and 4a show microscopic image of cells around the micro gap. Figs. 3b and 4b show velocity tracings of each cell. In Fig. 3b, cell enters the gap at $t = 0.5$ s, and apart from the gap at $t = 1.9$ s. Before $t = 0.4$ s, cell stops at the step of the entrance of the gap. In Fig. 4b, cell enters the gap at $t = 0$ s, and apart from the gap at $t = 0.45$ s. Figs. 3c and 4c show the projected two-dimensional area (S) tracings in the gap. The area S increases to the double value with the deformation of the cell in the gap. The cell in Fig. 4c, which has bigger S before the gap than the cell in Fig. 3c, passed through the gap with less increase of S than the cell in Fig. 3c. Which can show the different property according to each cell: deformation and friction behavior in the gap.

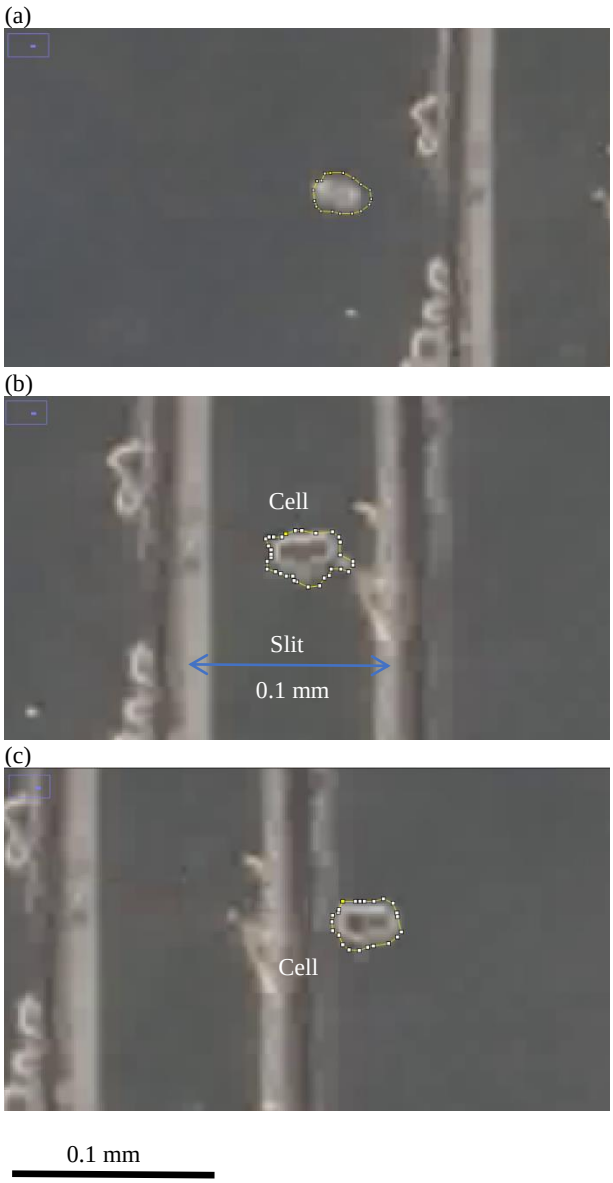

Fig. 1: Trace of contour of cell (No. 4) passing through slit: before slit (a), in slit (b), after slit (c): medium flows from left to right.

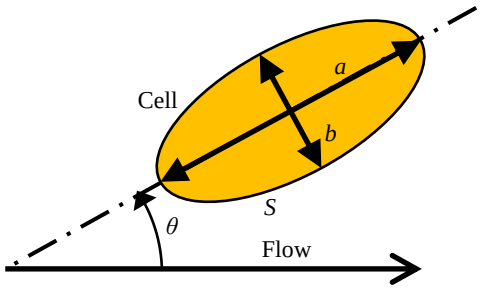

Fig. 2: Contour of cell was approximated to ellipsoid.

Figs. 3d and 4d show the shape index (P) tracings in the gap. Figs. 3e and 4e show the angle θ between the major axis and the medium flow direction in the gap. Both P and θ change in the gap, especially during the stop of the cell in the gap. The stepwise change of the angle occurs at the timing of the intermittent movement of the cell (Fig. 3b). When a cell passes through the slit, several cells repeat intermittent movement with the deformation. Each dotted line shows the regression line of data with the correlation coefficient (r). The projected area changes, the shape index changes, and the direction of the major axis changes.

Figs. 3f and 4f show the relationships between the shape index P and the area S. Figs. 3g and 4g show the relationships between the angle $\Delta\theta$ and the area S. Figs. 3h and 4h show the relationships between the shape index P and the angle $\Delta\theta$. The absolute acute values of θ are used for the angle in Figs. 3g, 3h, 4g, 4h. The shape index P tends to increase with the decrease of the area S (Figs. 3f and 4f).

Fig. 3a: Cell (No. 4).

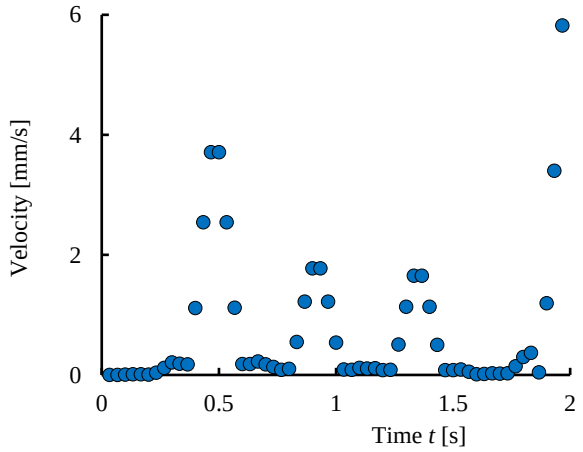

Fig. 3b: Velocity tracings of cell (No. 4).

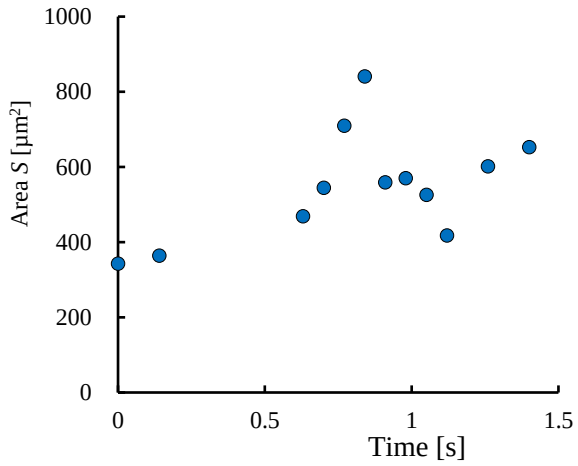

Fig. 3c: Area tracings of cell (No. 4).

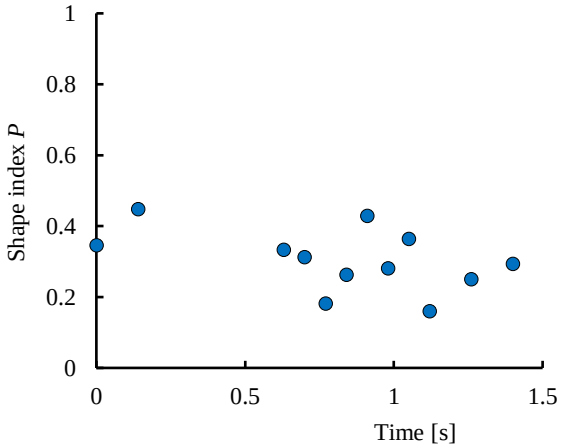

Fig. 3d: Shape index tracings of cell (No. 4).

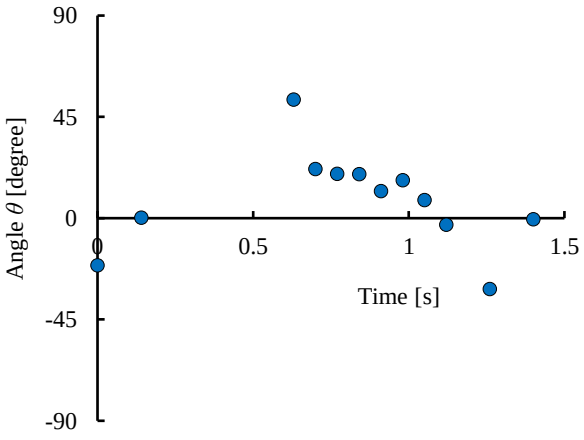

Fig. 3e: Angle (θ) tracings of cell (No. 4).

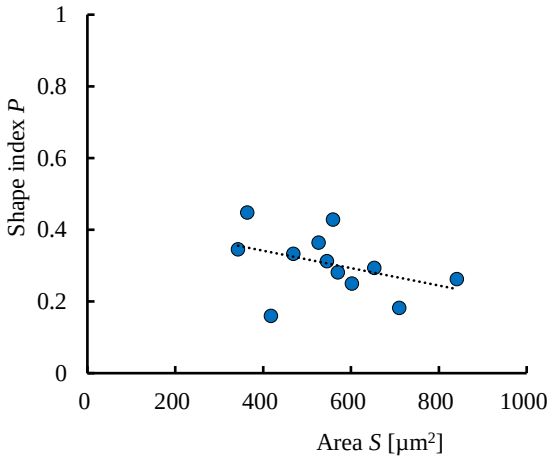

Fig. 3f: Shape index P vs. area S (No. 4): dotted line $P = -0.0002\ S + 0.44$, $r = 0.40$.

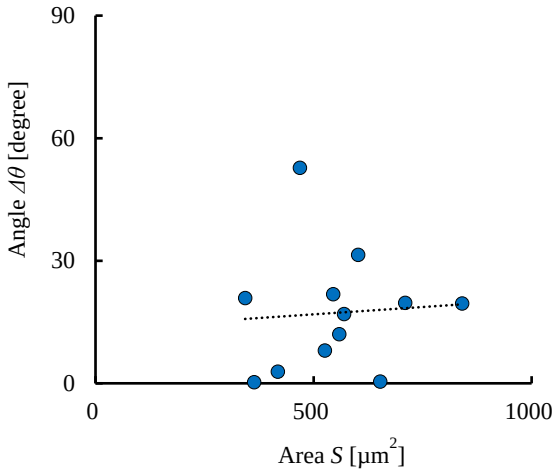

Fig. 3g: Angle $\Delta\theta$ vs. area S (No. 4): dotted line $\Delta\theta = 0.0072\ S + 13$, $r = 0.07$.

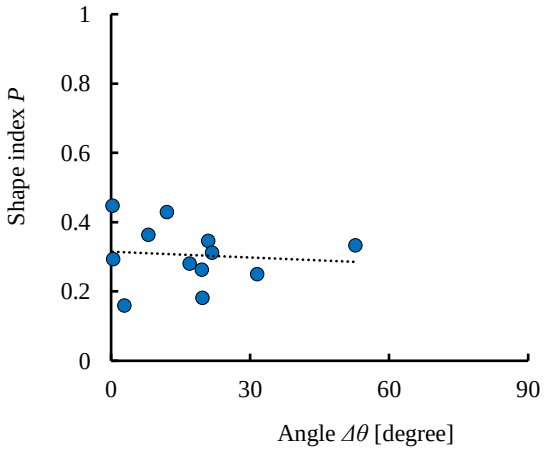

Fig. 3h: Shape index P vs. angle $\Delta\theta$ (No. 21): dotted line $P = -0.0006\ \Delta\theta + 0.31$, $r = 0.09$.

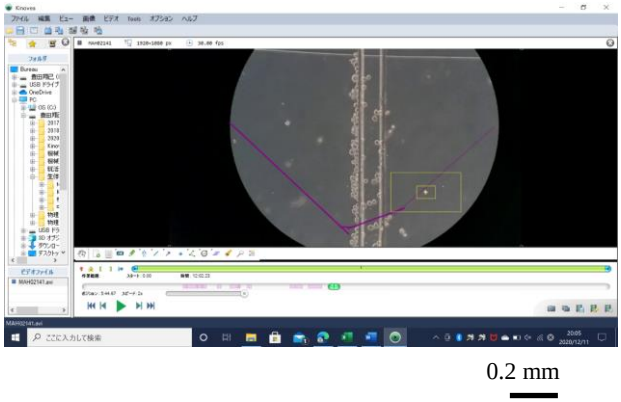

Fig. 4a: Cell (No. 21) passing through slit.: cell flows left to right.

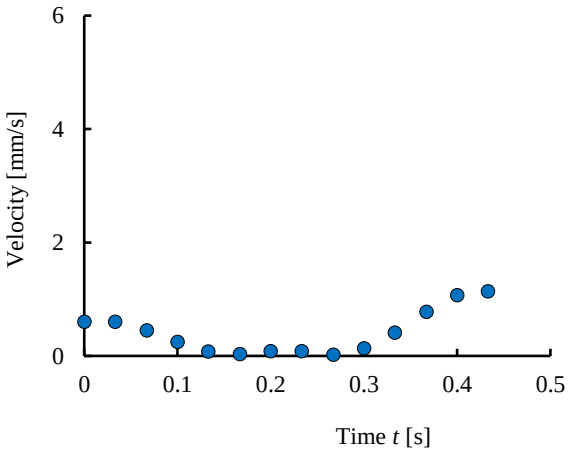

Fig. 4b: Velocity of cell (No. 21) passing through slit.

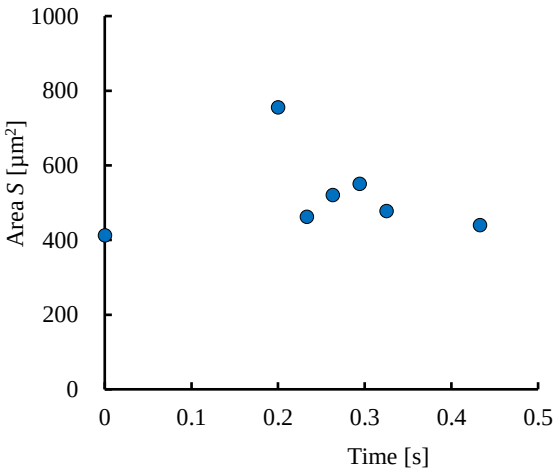

Fig. 4c: Area of cell (No. 21) passing through slit.

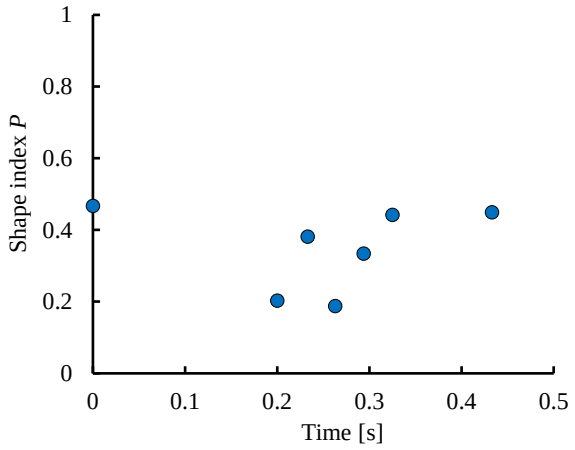

Fig. 4d: Shape index of cell (No. 21) passing through slit.

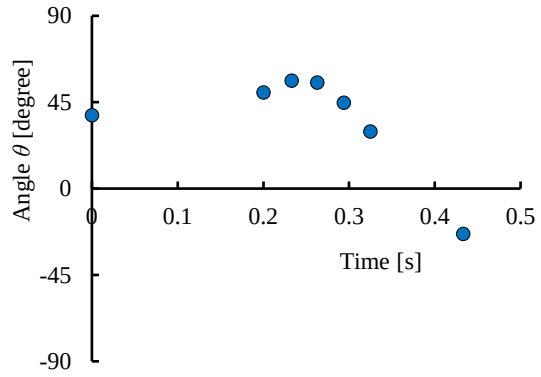

Fig. 4e: Angle of cell (No. 21) passing through slit.

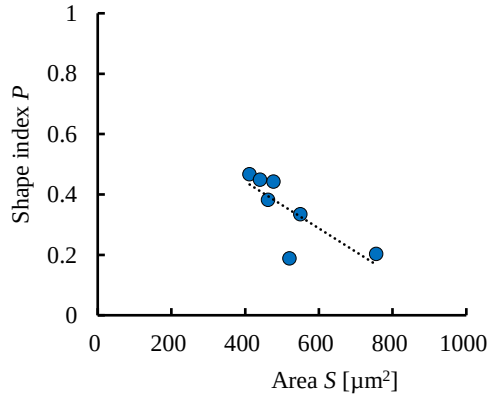

Fig. 4f: Shape index P vs. area S (No. 21): dotted line $P = -0.0008\,S + 0.75$, $r = 0.77$.

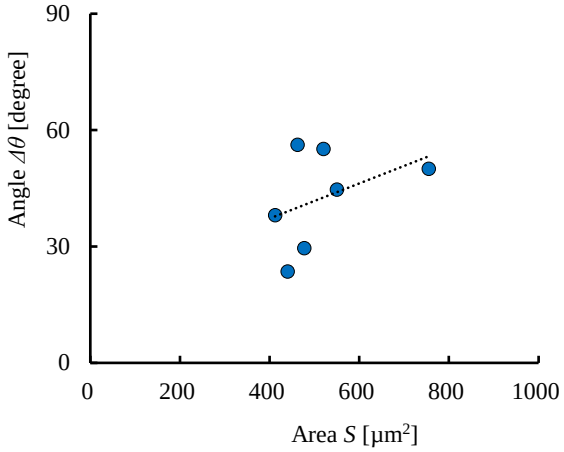

Fig. 4g: Angle $\Delta\theta$ vs. area S (No. 21): dotted line $\Delta\theta = 0.045\,S + 19$, $r = 0.41$.

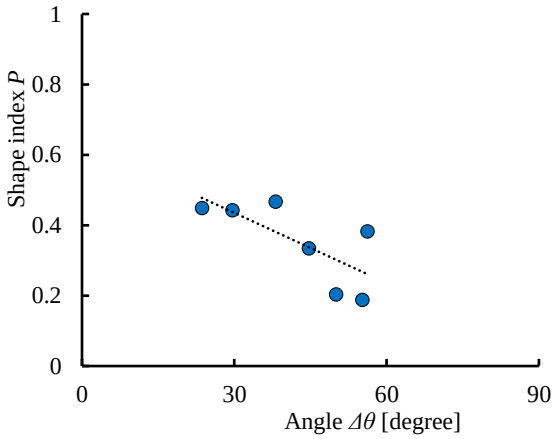

Fig. 4h: Shape index P vs. angle $\Delta\theta$ (No. 21): dotted line $P = -0.0066\,\Delta\theta + 0.63$, $r = 0.72$.

4. DISCUSSION

For the reason of the limitation of the photolithography process, the edge of the ridge is not sharp, but it has the edge with the small width. The biological system might have the sharper edge, so that a cell passes easily through the slit with the shorter travel distance *in vivo* [5]. The cell has to struggle to pass through the slit in the present experimental device with the longer travel distance.

The dimension of the slit was confirmed by the passing velocity of porcine red blood cells in the present study. The ratio of the cross-sectional area between the flow channel (0.06 mm × 2 mm) and the slit (0.01 mm × 0.8 mm) is 15, so that the mean flow velocity of the media in the slit is 15 times faster than that before the slit. In the present experiment, every datum of the velocity ratio was smaller than 5, which depends on the friction between the cell and the wall of the slit.

The moving velocity of the cell suspended in the media follows the media velocity. In the previous study [10], the flow rate was controlled by the syringe pump, but the flow rate varied because of several factors: the compliance of the wall of the flow path, and clogging of the flow path.

The flow rate is controlled by the pressure difference between inlet and outlet of the flow channel in the present study, which has advantage to keep the inner pressure of the flow channel for the morphological stability of the flow channel. The moving velocity also depends on the interaction between the cell and the surface of the slit (friction). To keep the surface property of the channel stable, bovine serum albumin is pre-coated on the surface of the flow path by prefilling the bovine serum albumin solution in the channel in the present study.

The maximum projected area of the cell, which flows in the channel is 250 μm^2 in the present experiment. The sphere with the diameter of 18 μm makes the projected circle area of 250 μm^2. Because the projected area is larger than 250 μm^2 in the slit, every cell is forced to be deformed from the sphere in the slit of 10 μm height in the present study. The biological cells are sorted according to the shape, and deformability *in vivo*. Several cells pass through the micro slit. Some cells or fragments, which pass through the slit, are decomposed. Some cells, which cannot pass through the narrow channel, are, on the other hand, captured at the channel.

A red blood cell has a high deformability. It deforms from the biconcave disk shape to the parachute like shape, when it is passing through the micro capillary. In the shear field, the red blood cell rotates and deforms to the ellipsoid shape. The most of biological cells, on the other hand, keep the spherical shape, when they are flowing in the medium. The deformability of a red blood cell changes with aging [3].

The deformability of the biological single cell depends on several factors. The deformability has been analyzed in several studies: using microfluidics [9], measuring local viscoelasticity [12], using atomic force microscopy [13, 14], and using cell mechanics model [15]. The methodology can also be applied to the soring technology on cells [16, 17]. The deformation is evaluated with the ratio of the projected area of the plan view of the disk-like shape during the passing through the slit in the present study. The deformation in the perpendicular direction can be observed at another type of the slit between micro cylindrical pillars [10].

During the intermittent movement of each cell passing through the slit, the cell shows deformation and rotation. Elongation of the cell tends to decrease the area of the cell. The designed micro slit can be used to analyze the mechanism of deformation of the single biological cell to pass through the silt.

5. CONCLUSIONS

During the intermittent movement of each cell passing through the slit, the cell shows deformation and rotation. Elongation of the cell tends to decrease the area of the cell facing the wall of the gap.

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The authors thank to Dr. Yusuke Takahashi and Mr. Yuki Takiguchi for their assistance of the experiment.

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Effect of Shape of Cell on Dielectrophoretic Movement in Flow Channel

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ABSTRACT

The effect of the shape of the flowing single cell on dielectrophoretic movement in a flow channel has been investigated *in vitro*. A pair of asymmetric surface electrodes of titanium were manufactured by the photolithography technique: a triangular electrode with the tip angle of 0.35 rad, and a rectangular electrode of the flat edge as the reference. The cyclic alternating electric current of the square wave (between 0.25 μs and 0.3 μs of periods) was introduced between the surface electrodes, to induce the asymmetric electric field perpendicular to the main flow direction. The suspension of cells (C2C12: mouse myoblast cell line) was injected into the flow channel, and the flow rate was controlled by the pressure head between the inlet and the outlet. The experimental result shows that the absolute value of the amplitude of the acceleration by the electric field, which is perpendicular to the flow direction, depends on the shape of the floating cell.

Keywords: Biomedical Engineering, Dielectrophoresis, Flow Channel, Shape Index, Surface Electrode and C2C12.

1. INTRODUCTION

The movement of a biological cell suspended in the medium is governed by several factors: movement of the medium, gravity, electric force, Van der Waals force, and affinity of surface. Various methods have been applied to control the movement of cells i*n vitro*: the flow [1,2], the shear field, the filter [3], the slit, the magnetic field, the gravitational field, laser and the electric field. These methods might contribute to several applications of manipulation of cells [4,5]: detection of targeted cells [6], sorting of cells [7], arrangement of cells to make a tissue, and measurement of the character of cells [8,9].

Movement of a charged particle depends on the electric field. The effect is applied to the electrophoresis device. When a particle is subjected to a non-uniform electric field, a force acts even on a non-charged particle. The effect depends on the polarization generated in the particle. The phenomenon is called dielectrophoresis [10]. The force of dielectrophoresis depends on the several parameters: the electrical property of the particle, the shape and the size of the particle [11], the electrical property of the medium, and the electric field (the amplitude and the frequency). The effect is also observed at the cluster of cells [12].

The dielectrophoretic force $\boldsymbol{F}$ is generated by the nonuniform electric field $\boldsymbol{E}$.

$$\boldsymbol{F} = -2\pi\varepsilon (d/2)^3 \boldsymbol{E} \nabla \boldsymbol{E} \quad (1)$$

In Eq. (1), d is the diameter of the particle, and ε is the liquid permittivity.

In the present study, the effect of the shape of the flowing single cell on dielectrophoretic movement in a flow channel has been investigated *in vitro*.

2. METHODS

Electric Stimulation
The electric stimulation of the alternating rectangular cyclic wave (0.25 μs < period (T) < 0.3 μs; −15 V < amplitude (Ea) < +15 V) was generated with an electric stimulator. The stimulator was connected to the titanium film electrode, and the electric stimulation was applied to the medium flow. An electric resistance (R) of 2 kΩ is serially inserted between the electrode and the stimulator to measure the electric current (I < ±7.5 mA).

Cell
C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse) was used in the test. D-MEM (Dulbecco's Modified Eagle Medium) containing 10% FBS (Fetal Bovine Serum) and 1% penicillin/ streptomycin was used for the medium.

Before the flow test, the inner surface of the flow channel was hydrophilized by the oxygen plasma ashing. The bovine serum albumin solution was prefilled in the flow channel, and was incubated for thirty minutes at 310 K in the incubator.

Before the flow test, the cells were exfoliated from the plate of the culture dish with trypsin including EDTA (ethylenediaminetetraacetic acid), and were suspended in the medium. The suspension of cells was poured at the inlet of the flow channel. The flow was made by the pressure difference between the inlet and the outlet, which was kept by the gravitational level of the medium (< 5 mm).

Each cell passing between the electrodes was observed by the inverted phase-contrast microscope, and recorded by the video camera, which is set at the eyepiece of the microscope.

Movement Analysis

The movement of the cell was analyzed by "Kinovea (Ver. 8.23, Commons Attribution)" at the video images: 30 frames per second. To trace the movement of the cell, the coordinates are defined as that in Fig. 1. The main flow direction of the medium is defined as x. The perpendicular direction from the reference electrode to the tip of the triangular electrode is defined as y. The origin is adjusted at the tip of the triangular electrode. The components (v_x, and v_y) of velocity were calculated at the tracings of each cell. The components (a_x, and a_y) of acceleration of the velocity were calculated at the velocity tracings of each cell.

The contour was approximated to the ellipsoid. On the ellipsoid, the length of the major axis (f_1), and the length of the minor axis (f_2) were measured. The ratio between axes is calculated as the shape index (f) by Eq. (2).

$$f = 1 - f_2 / f_1 \tag{2}$$

At the circle, $f = 0$. As the ellipsoid is elongated, f approaches to one.

3. RESULTS

Fig. 2 shows the trace of each cell: under the electric pulses with the period of 0.25 μs (Fig. 2a) and 0.3 μs (Fig. 2b), respectively. Each cell moves stepwise around the tip of the electrode: around the origin ($x = 0$, $y = 0$). The change of the movement in y direction is maximum at the origin. The step is bigger at the period of 0.25 μs (Fig. 2a) than at the period of 0.3 μs (Fig. 2b). Both the velocity and the acceleration of the movement of each cell in the video image have been calculated from each tracing using Kinovea.

Fig. 3 shows shape index tracings at the electric pulse stimulation with the period of 0.25 μs. The shape index varies at random during passing through the electric fields in some cells. Each cell makes rotation and deformation, while moving with the medium. Four shape index tracings are exemplified in Fig. 3. One cell has the stable larger shape index (rhombus in Fig. 3). The shape index of another cell varies between 0 (circle in two-dimensional projection) and 0.7, which corresponds to the deformation between the sphere and the ellipsoid.

Figs. 4a and 4b show the relationships between the step of y component of movement of cell (Δy) and the shape index (f): under the electric pulses with the period of 0.25 μs (Fig. 4a) and 0.3 μs (Fig. 4b), respectively. The dotted line is the regression line of data in each figure: r is the correlation coefficient. The higher step Δy occurs between the shape index of 0.1 and 0.3, especially under the electric pulses with the period of 0.25 μs (Fig. 4a). The step (Δy) tends to decrease with the increase of the shape index (f).

Figs. 5a and 5b show the relationships between the acceleration in the y direction (a_y) and the shape index (f): under the electric pulses with the period of 0.25 μs (Fig. 5a) and 0.3 μs (Fig. 5b), respectively. The absolute value of the acceleration in the y direction (a_y) is small at the higher shape index (f). The higher acceleration in the y direction (a_y) occurs between the shape index of 0.1 and 0.2, especially under the electric pulses with the period of 0.25 μs (Fig. 5a).

Figs. 6a and 6b show the relationships between the acceleration difference in the y direction (Δa_y) and the shape index (f): under the electric pulses with the period of 0.25 μs (Fig. 6a) and 0.3 μs (Fig. 6b), respectively. The acceleration difference in the y direction (Δa_y) is calculated by Eq (3).

$$\Delta a_y = a_{ymax} - a_{ymin} \tag{3}$$

In Eq. (3), a_{ymax} and a_{ymin} are the maximum value of a_y, and the minimum value of a_y, respectively. The minimum value (a_{ymin}) occurs before passing near the tip of the electrode. The maximum value (a_{ymax}) occurs after passing near the tip of the electrode (Figs. 1 and 2). The higher acceleration difference in the y direction (Δa_y) occurs between the shape index of 0.1 and 0.3, especially under the electric pulses with the period of 0.25 μs (Fig. 6a).

Figs. 7a and 7b show the relationships between the maximum acceleration in the y direction (Δa_{ymax}) and the acute angle of the major axis of the cell (θ): under the electric pulses with the period of 0.25 μs (Fig. 7a) and 0.3 μs (Fig. 7b), respectively. The acceleration tends to decrease with increase of the angle, especially under the electric pulses with the period of 0.25 μs (Fig. 7a). The stepwise movement tends to be accelerated when the cell elongated to the flow direction.

Figs. 8a and 8b show the relationships between the maximum acceleration in y direction (Δa_{ymax}) and the length of the wrapping contour of cell in y-direction (y_c): under the electric pulses with the period of 0.25 μs (Fig. 8a) and 0.3 μs (Fig. 8b), respectively. The length of the wrapping contour of cell in y-direction does not affect the acceleration in y direction.

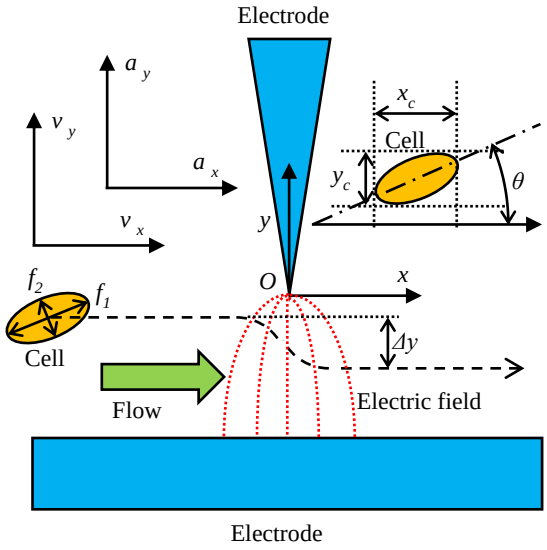

Fig. 1: Coordinate for description of movement of cell around electrodes.

Figs. 9a and 9b show the relationships among the maximum acceleration in y direction (Δa_{ymax}), the initial position of the cell (y_0) and the shape index (f): under the electric pulses with the period of 0.25 μs (Fig. 9a) and 0.3 μs (Fig. 9b), respectively. The initial position (y_0) is measured at $x = -0.5$ mm. The maximum acceleration is high around 0.2 of the shape index (f) and 0 of the initial position of y_0.

Figs. 10a and 10b show the relationships among the step of y component of movement of the cell (Δy), the initial position of the cell (y_0) and the shape index (f): under the electric pulses with the period of 0.25 μs (Fig. 10a) and 0.3 μs (Fig. 10b), respectively. The initial position (y_0) is measured at $x = -0.5$ mm. The step of Δy is high around 0.2 of the shape index (f) and 0 of the initial position of y_0.

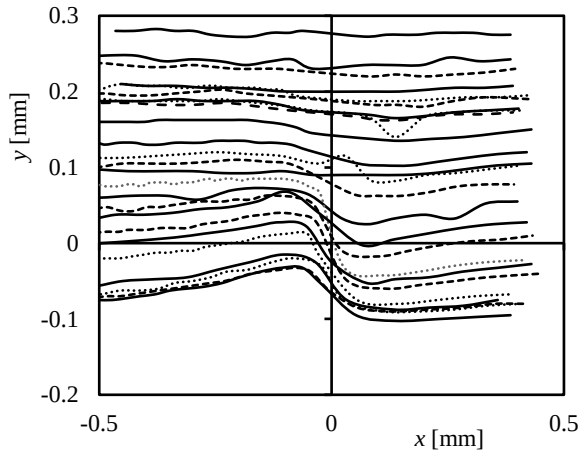

Fig. 2a: Movement of 27 cells: period of 0.25 μs.

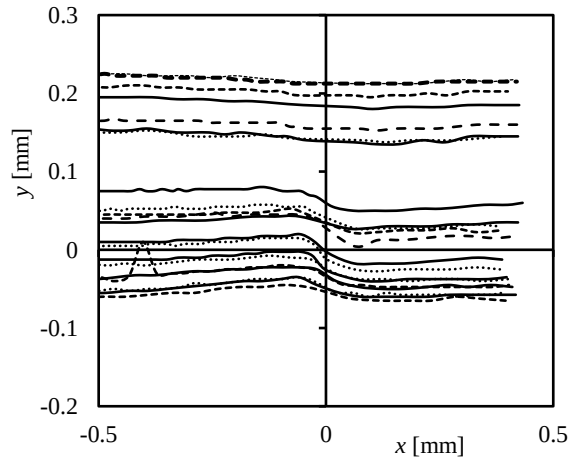

Fig. 2b: Movement of 21 cells: period of 0.3 μs.

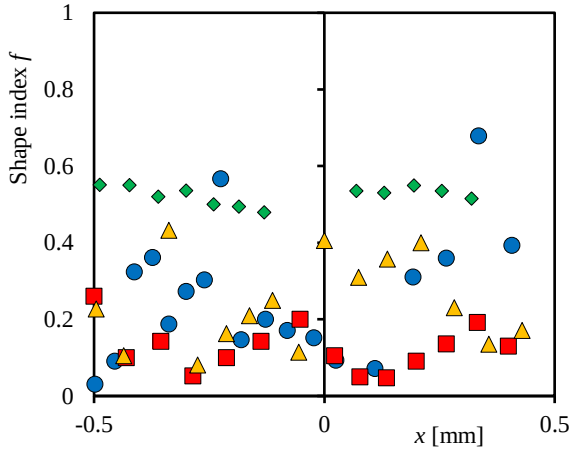

Fig. 3: Shape index tracings of four cells: period of 0.25 μs.

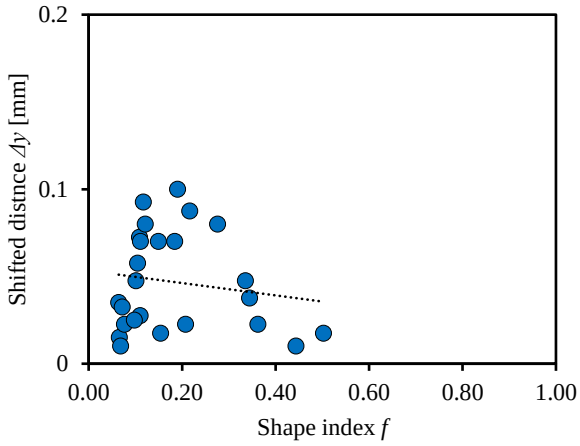

Fig. 4a: Relationship between shifted distance (Δy) and shape index (f): period of 0.25 μs: dotted line, $\Delta y = -0.035 f + 0.024$, $r = 0.15$

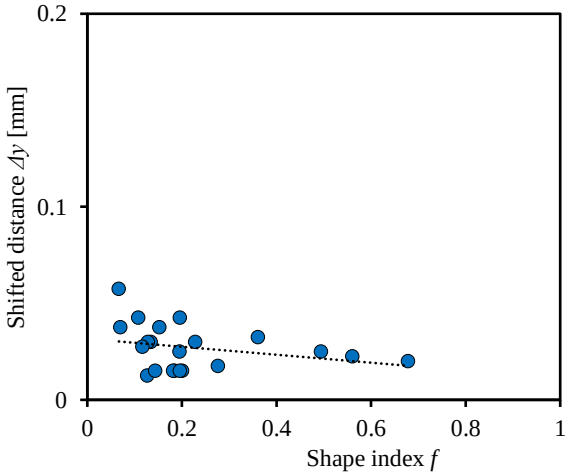

Fig. 4b: Relationship between shifted distance (Δy) and shape index (f): period of 0.3 μs: dotted line, $\Delta y = -0.021 f + 0.032$, $r = 0.28$.

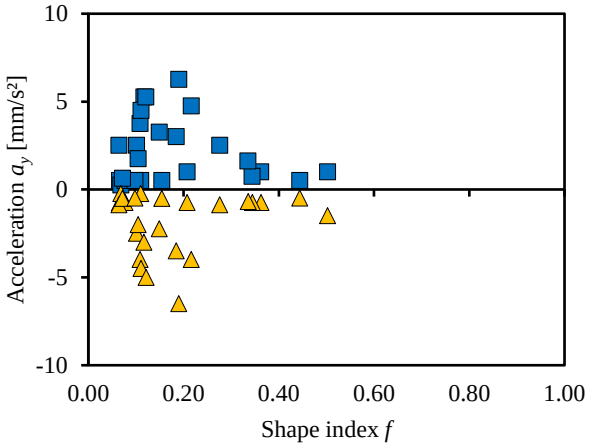

Fig. 5a: Relationship between acceleration (a_y) and shape index (f): period of 0.25 μs.

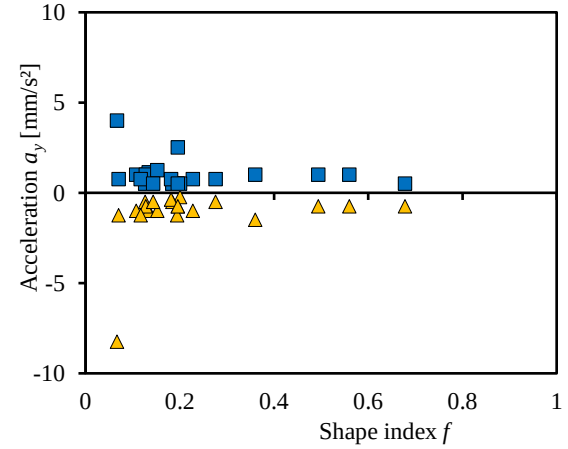

Fig. 5b: Relationship between acceleration (a_y) and shape index (f): period of 0.3 μs, amplitude of ±15 V.

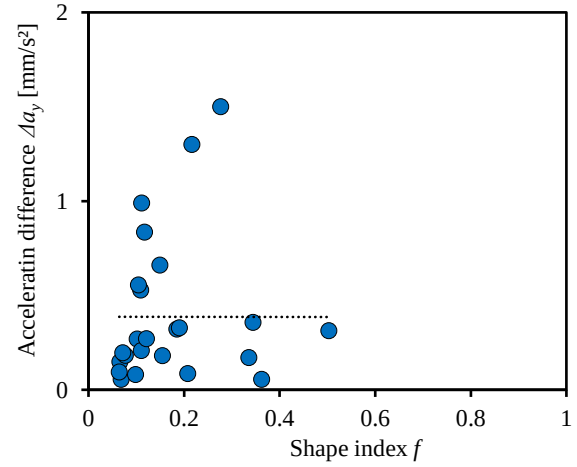

Fig. 6a: Relationship between acceleration difference (Δa_y) and shape index (f): period of 0.25 μs: dotted line, $\Delta a_y = -0.003\, f + 0.38$, $r = 0.0009$.

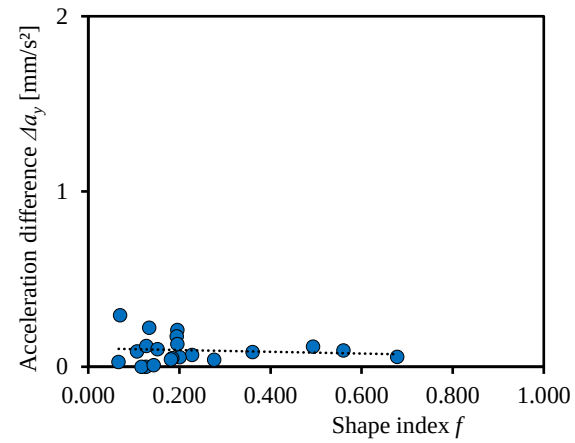

Fig. 6b: Relationship between acceleration difference (Δa_y) and shape index (f): period of 0.3 μs: dotted line, $\Delta a_y = -0.05\, f + 0.11$, $r = 0.11$.

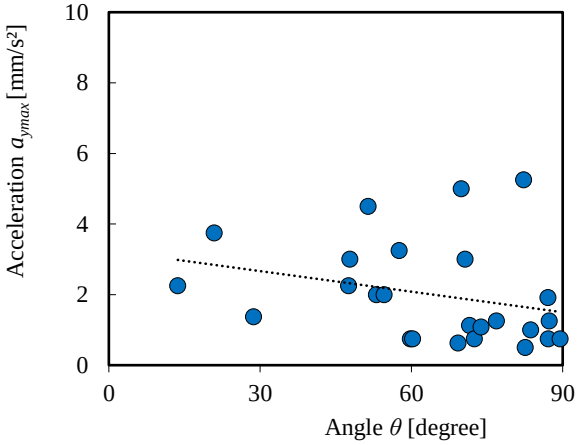

Fig. 7a: Relationship between maximum acceleration (Δa_{ymax}) and angle (θ): period of 0.25 μs: dotted line, $a_{ymax} = -0.019\, \theta + 3.25$, $r = 0.28$.

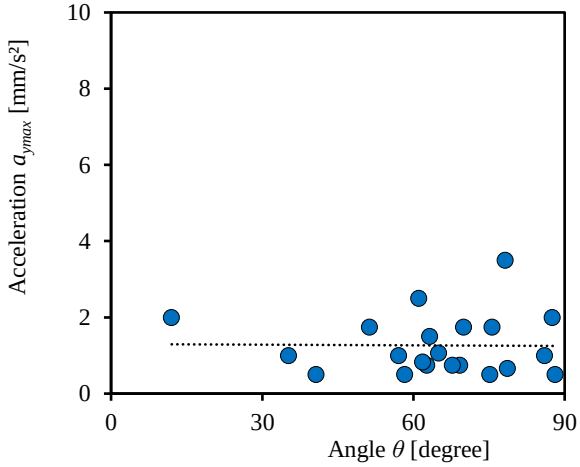

Fig. 7b: Relationship between maximum acceleration (Δa_{ymax}) and acute angle (θ): period of 0.3 μs: dotted line, $a_{ymax} = -0.0006\, \theta + 1.3$, $r = 0.01$.

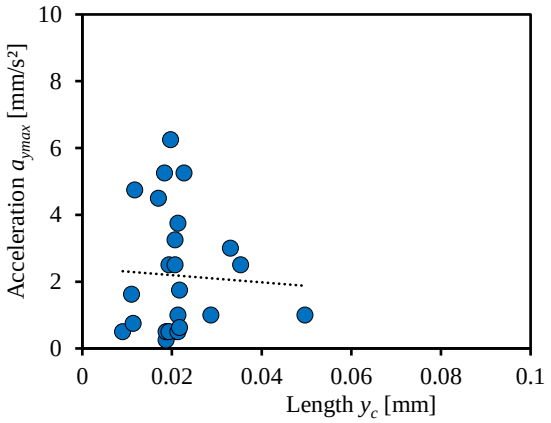

Fig. 8a: Relationship between maximum acceleration (Δa_{ymax}) and length (y_c): period of 0.25 μs: dotted line, $a_{ymax} = -10.7\ y_c + 2.41$, $r = 0.05$.

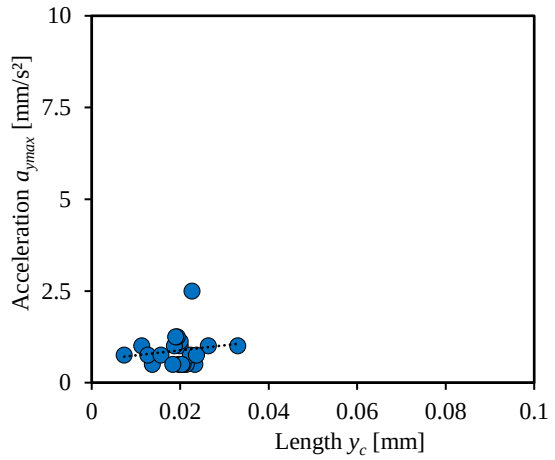

Fig. 8b: Relationship between maximum acceleration (Δa_{ymax}) and length (y_c): period of 0.3 μs: dotted line, $a_{ymax} = 14\ y_c + 0.6$, $r = 0.16$

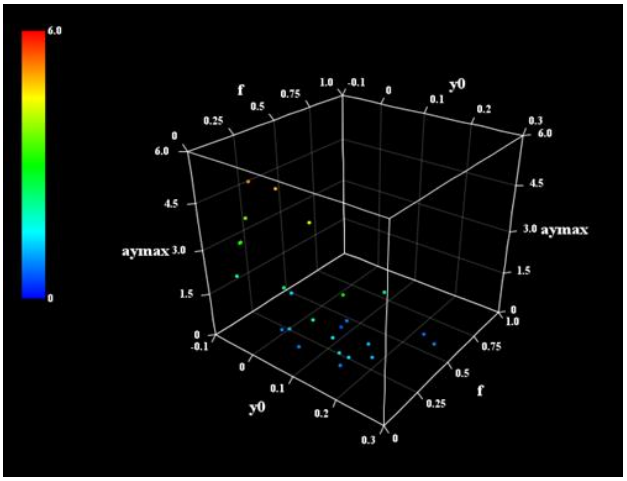

Fig. 9a: Relationship among maximum acceleration (a_{ymax}), shape index (f), and initial position (y_0): period of 0.25 μs, amplitude of ±15 V.

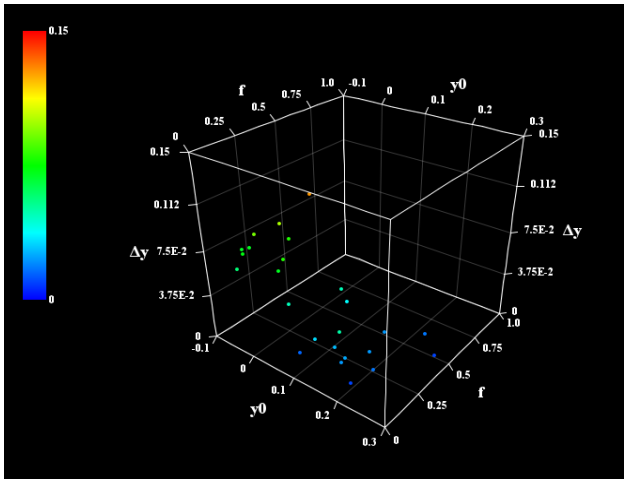

Fig. 9b: Relationship among shifted distance (Δy), shape index (f), and initial position (y_0): period of 0.25 μs, amplitude of ±15 V.

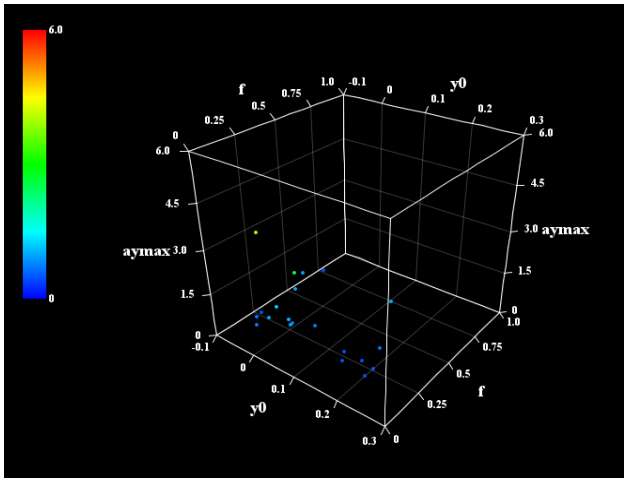

Fig. 10a: Relationship among maximum acceleration (a_{ymax}), shape index (f), and initial position (y_0): period of 0.3 μs, amplitude of ±15 V.

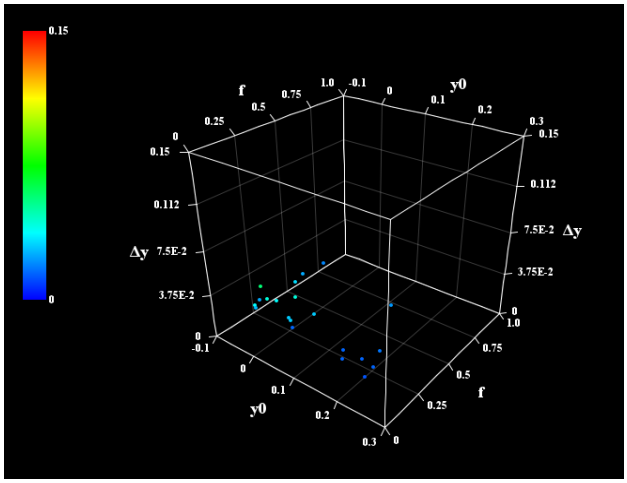

Fig. 10b: Relationship among shifted distance (Δy), shape index (f), and initial position (y_0): period of 0.3 μs, amplitude of ±15 V.

4. DISCUSSION

Several forces can be applied to the flowing cell during passing through the electric field in the flow channel: the resistant force against the movement of the flowing cell by the flow of the medium, the resistant force against the movement by the viscosity of the medium, and dielectrophoretic force. When the cell is deformed to ellipsoid ($f > 0.4$), the viscous resistance might make the smaller movement perpendicular to the flow direction. The mechanism can be applied to sorting cells according to deformability.

The deformation of each cell during the movement along the flow can cause the change of the dielectrophoretic force passing through the electric field. The dielectrophoretic movement mainly depends on the shape index of the cell at the timing when the cell is passing near the tip of the electrode (Figs. 9 and 10). The fluctuation of the dielectrophoretic movement of each cell relates to the fluctuation of the deformation of the cell.

The cell movement perpendicular to the flow of the medium depends on the position of the route through which the cell flows. The maximum movement perpendicular to the flow direction occurs at the route passing near the tip of the electrode. The shifted distance depends on the passing route in the electric field, because nonuniform electric field is necessary for dielectrophoretic movement. The three-dimensional display (Figs. 9 and 10) shows the dependency of the shifted distance (Δy) on y_0. The shifted distance (Δy) also depends on the velocity of the cell [13].

The gap of *z*-direction, which is perpendicular to the *x*-*y* plane, is small (35 μm) in the flow channel of the present study. A nozzle type of the guide at the upstream of the flow channel might be effective to adjust the initial position of the cell. For the more precise analysis, three-dimensional information of cell is necessary. In the present study, only two-dimensional projected shape is analyzed. The rotation of the cell can display the change of the shape index. The effect of dielectrophoresis can vary by the rotation of the cell with the asymmetric shape.

5. CONCLUSIONS

The experimental result shows that the absolute value of the amplitude of the acceleration by the electric field, which is perpendicular to the flow direction, depends on the shape of the floating cell: the maximum acceleration occurs at the shape slightly (10 %) deformed from the sphere (*f* around 0.1).

ACKNOWLEDGMENT

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Earphone Hearing Loss
- Discussion of Accuracy of Ear Age Conversion Method -

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ABSTRACT

In our laboratory, ear age is used to evaluate the degree of exhaustion for basic research on the mechanism of earphone hearing loss. The averaged audiogram as the standard to the evaluation is needed. As many audiograms as possible must be collected and the population can be statistically obtained

In this study, Japanese and foreign literature was searched, a large number of audiograms were collected, and their averaging was performed. The variability was large, and there were not few documents in which the subject information was not clear, or which the reference sound pressure was changed. Average audiograms for Japanese women, Japanese men, foreign women, and foreign men were obtained. In the future, we will evaluate the error and aim to improve the accuracy of ear age estimation.

Keywords: Prediction of ear age, Averaging audiograms, Sound pressure, Hearing level, Statistic analysis.

1. INTRODUCTION

In our laboratory, the authors are conducting basic research on the mechanism of earphone hearing loss [42] for the purpose of preventing and enlightening earphone hearing loss, which has become a serious issue for young people worldwide in recent years. The more you listen to the sound, the more your ears are exhausted. Ear age is good evaluation to be used as the degree of exhaustion. Improving the accuracy of ear age calculation is one of the important issues in this study.

An audiogram is needed when assessing ear age. Since the subjects are different, the audiograms differ depending on the publishing organization. It must be that only one audiogram should not be used as it is for estimating ear age. On the other hand, there is no audiogram based on the hearing of all the people over the world. The ultimate goal of this study is to estimate the population of audiograms. To accurately estimate the ear age of the subject, as many audiograms as possible should be collected and be statistically processed.

In this paper, the results obtained by averaging the audiograms collected so far are described. The averaging method, the characteristics of the averaging audiogram, and matters to be notes due to the error when using it for ear estimation will be discussed.

2. EVALUATION OF HEARING SOUND

Evaluation of Sound
The previous findings regarding quantitative evaluation of sound are describes as follows;

Intensity of sound: Intensity of sound [J] is defined as the energy of the sound. It is a physical quantity and is proportional to the square of sound pressure.

Sound pressure: Sound pressure [Pa] is defined as the fluctuation of pressure from atmospheric pressure when the sound propagates in the air. Specifically, the air pressure P also changes by ΔP when the density of the air ρ changes by $\Delta\rho$ due to the sound wave. Here, ΔP is called sound pressure.

Sound pressure level, SPL: Sound pressure level, P_{SPL} [dB] is defined by the following Eq. (1) for the sound pressure ΔP [Pa]. It is a logarithmic representation of ΔP.

$$P_{SPL} = 10 \times \log_{10}\left(\frac{\Delta P}{(\Delta P)_0}\right)^2 = 20 \times \log_{10}\left(\frac{\Delta P}{(\Delta P)_0}\right) \quad \cdots \quad (1)$$

Here, the decibel reference amount $(\Delta P)_0$ is defined as 20μPa which value was once the minimum audible sound pressure that humans can hear at 1 kHz [43].

Loudness: Loudness [sone] is how loud humans feel the sound. It is the psychology quantity, and depends on a person. Furthermore, when the frequencies are different, it is not always possible to discuss the magnitude of loudness by the magnitude of sound pressure level because the human hearing shows different sensitivities depending on the frequency.

Loudness level: Then, the weight according to the frequency with reference to 1kHz, which can compare the loudness of sounds with different frequencies. Here, the sound pressure level of a 1kHz pure tone which people feel to be the same loudness of the sound is called the loudness level [phon], whose graphic display is called the equal-loudness contours.

Hearing level: Hearing is evaluated by quantifying how much you can hear, based on the sound a healthy person should be able to hear. Hearing level P_{HL} when the subject can hear up to the volume ΔP is defined by the following Eq. (2) based on the lowest sound pressure $(\Delta P)_0$ a healthy person can hear.

$$P_{HL} = \log_{10}\left(\frac{\Delta P}{(\Delta P)_0}\right) = \frac{1}{20} P_{SPL} \quad \cdots\cdots\cdots\cdots\cdots\cdots \quad (2)$$

The maximum value of P_{HL} is 120dB. It is normal at less than 20dB, symptoms with a hearing level of 30±10dB is called mild deafness (mildly hard of hearing), and that of 50±10 dB, 80±10dB, and more than 90dB are called moderate deafness, severe deafness, and deaf, respectively.

Average Audiogram

A graph showing the relationship between the frequency of the sound the subject can hear and his hearing level as a curve for each age is called an audiogram, with sound frequency [Hz] on the horizontal axis and hearing level [dB] on the vertical axis. It can be created by investigating the hearing level of the recruited subjects. Different audiograms are to come out from different subjects. Therefore, when a subject's hearing (hearing level) is discussed based on a certain audiogram, the audiogram should be average.

In our laboratory, the authors use the average audiogram drawn in 2012, which is as shown in Fig. 1 [42] and was obtained by referring to several documents presented in Japan between 2010 and 2012, in order to estimate the ear age of the subjects. The dashed lines mean that only a small amount of data could be referred to. They were drawn so that each curve itself was smooth and all the curves corresponding to each age are lined up smoothly as a whole. The audiogram is drawn as mixed gender data, and it is not sure if it can be applied to anyone other than Japanese. Also, the variability of the referenced audiograms was not recorded.

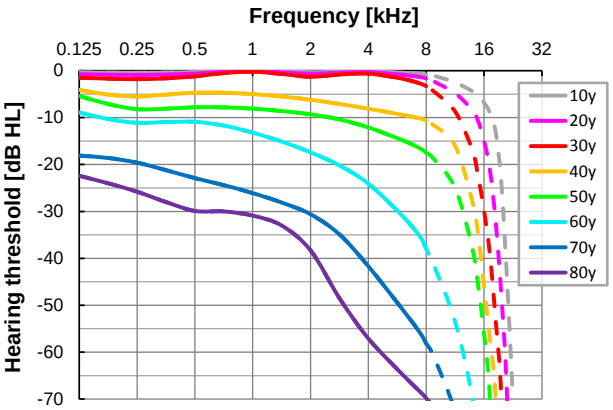


Fig. 1 : Employed audiogram in this laboratory.

It is preferable that the ear age can be estimated more accurately in consideration of the characteristics of the subject such as race and gender. For the purpose, the previous audiograms are to be collected again and an average audiogram will be drawn for each characteristic. The authors would like to estimate the population mean and population variance of the average audiogram corresponding to each characteristic, to evaluate the ear age estimation accuracy of each characteristic, and to discuss the differences in the audiograms that exist between the characteristics.

3. COLLECTION OF AUDIOGRAM

Essential Method to Average Data

In essence, the average curves should be created based on the data obtained from subjects with the same characteristics. Hearing level P_{HL} is only a "converted value" which clearly indicates the appearance of the phenomenon of hearing, so all data should be treated with the original data, sound pressure ΔP. From Eq. (2'), the original sound pressure $(\Delta P)_i$ corresponding to the hearing level read from the audiogram i can be calculated.

$$(\Delta P)_i = 10^{P_{HL,i}} \times (\Delta P)_0 \quad \cdots\cdots\cdots\cdots\cdots\cdots \quad (2')$$

By multiplying the obtained sound pressures $(\Delta P)_i$ by the numbers of people n_i as a weight and then averaging them, the average sound pressure $(\Delta P)_{mean}$ of the original data of multiple audiograms can be calculated as shown in Eq. (3).

$$(\Delta P)_{mean} = \frac{\sum_i n_i \cdot (\Delta P)_i}{\sum_i n_i} \quad \cdots\cdots\cdots\cdots\cdots\cdots \quad (3)$$

Converting this value to the hearing level again gives the average value $P_{HL,mean}$ as shown in Eq. (4).

$$P_{HL,mean} = \log_{10}\left(\frac{(\Delta P)_{mean}}{(\Delta P)_0}\right) \quad \cdots\cdots\cdots\cdots\cdots\cdots \quad (4)$$

The variance $(\Delta P)_{variance}$ and standard deviation $(\Delta P)_{deviation}$ can be calculated in the same way as shown in Eqs. (5) and (6), respectively.

$$(\Delta P)_{\mathrm{variance}} = \frac{\sum_i n_i \cdot \{(\Delta P)_i - (\Delta P)_{mean}\}^2}{\sum_i n_i} \quad \cdots\cdots \quad (5)$$

$$(\Delta P)_{deviation} = \sqrt{(\Delta P)_{\mathrm{variance}}} \quad \cdots\cdots\cdots\cdots\cdots\cdots \quad (6)$$

Converting the standard deviation $(\Delta P)_{deviation}$ to hearing level in the same way gives the standard deviation value $P_{HLdeviation}$ as shown in Eq. (7). The variance value $P_{HLvariancen}$ cannot

meaningfully be calculated because it is with a different unit.

$$P_{HL,deviation} = \log_{10}\left(\frac{(\Delta P)_{deviation}}{(\Delta P)_0}\right) \quad \cdots\cdots\cdots\cdots \quad (7)$$

List of Collected Audiograms

Table 1 shows the list of the collected audiogram with the number of the subjects and their characteristics. The No. indicates a reference number [1]-[41]. There were many unclear data on the subjects. Many data groups on Japanese people were obtained. On the other hand, about data for non-Japanese people, although the data group obtained was few, the element dimension of each data group was sufficiently large.

Table 1 : List of collected audiogram.

Augiogram		Subjects		
No.	When drawn	Number	Nationarity	Sex
1 - 3	1997, 1999, 2000	80	Japanese	mixed
4	unclear	60	Japanese	mixed
5	1992 - 2002	1521	Japanese	separated
6	2010 - 2012	148	Korean	separated
7	1984 -	1600	Japanese	separated
8	2004 - 2005	3470	Korean	separated
9	unclear	645	Spanish	mixed
10	2009 - 2013	1175	Spanish	separated
11	unclear	263	Korean	separated
12	unclear	165	Japanese	separated
13	unclear	334	Japanese	separated
14	unclear	1192	Japanese	separated
15	1987	239	Japanese	male only
16	unclear	120	Japanese	mixed
17	2006 - 2008	490	Japanese	separated
18	unclear	148	Italian	mixed
19	2006	52	Japanese	separated
20	unclear	90	Japanese	mixed
21	1990	6922	Japanese	separated
22	unclear	412	Japanese	mixed
23	unclear	546	Japanese	mixed
24	unclear	1175	Japanese	separated
25	2010 - 28 Feb. 2011	216	Japanese	mixed
26	2011 - 31 Jan. 2012	230	Japanese	mixed
27	8 July, 20 Sep. 2018	152	Japanese	mixed
28 - 33	2011 - 2016	2673	Japanese	separated
34	unclear	200	Japanese	separated
35	unclear	110	Chainese	mixed
36	1993 - 2005	3553	American	separated
37	unclear	83	Italian	mixed
38	1998 - 2005	346	Japanese	separated
39	unclear	42	Japanese	mixed
40	unclear	360	Japanese	mixed
41	2011 - 2015	4743	Dutch	separated

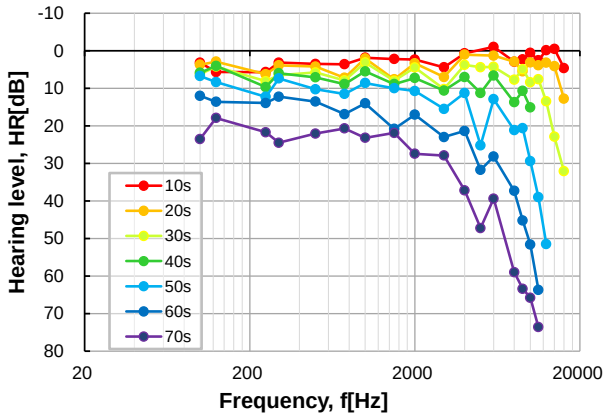
Realistic Method to Average Data

It was inferred that the reference sound pressures of the collected audiograms were different for each. This is because by translating the curves of the audiogram in the HL axis direction, the curves of the audiograms that were separated from each other matched fairly well. As a result, the reference sound pressure must be made uniform by translation before the statistical processing, and the method shown in the above equations (2') to (7) makes the averaging very complicated. In addition, in audiometry, data is often managed in unit dB from the beginning instead of sound pressure. Considering above, it may be reasonable to simply process the conversion value in dB instead of the original method to process the original value in MPa. When organizing the data related to the destruction of objects by the Weibull distribution, the distance to the destruction of the original data value (elapsed time or the number of cyclic loading) and the destruction probability are arranged by the logarithmic value. Following it, the data organization method is changed to the policy that P_{HL} is substituted in Eq. (3) instead of ΔP.

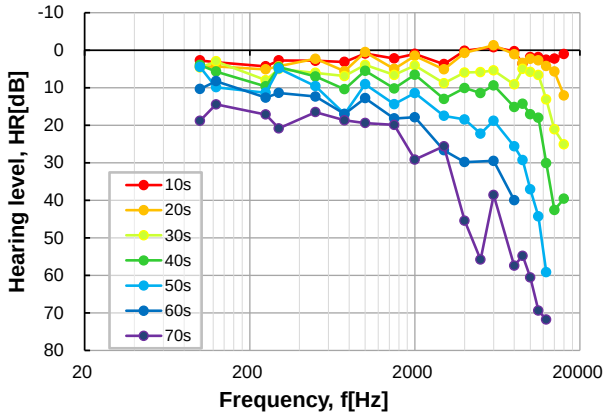
Results of Averaged Audiograms

First, the curves of the collected audiograms were translated along the HL axis so that they were generally closest to each other for all ages. The averaged audiograms were created for each gender, and other characteristics of the subjects were excluded from the classification this time.

Japanese data: Fig. 2 shows the Japanese averaged audiogram. There was no data below 100Hz. On the other hand, the energy of sounds below 200Hz is considered to be significantly smaller than the energy of higher sounds [44], so it may be that HR below 100Hz is almost constant regardless of age. The difference between female and male was not clear in the Japanese data. That is, it was suggested that the gender difference in hearing may be negligible for healthy people.



(a) Female

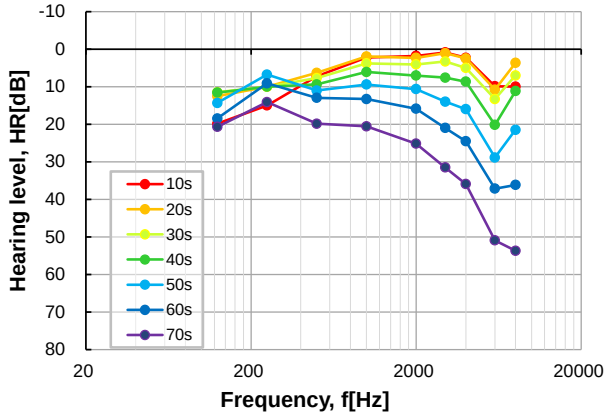


(b) Male

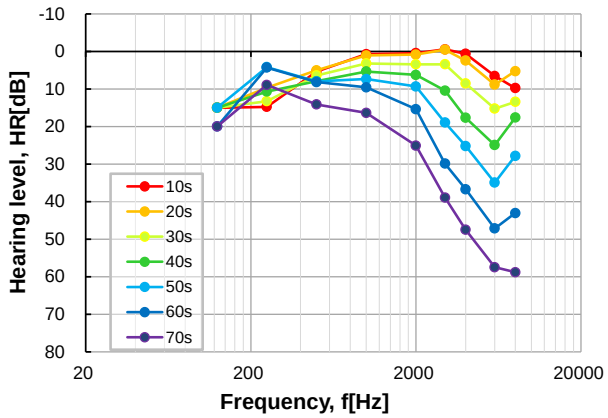
Fig.2 : Japanese averaged audiogram.

However, it seems that there are more male hearing loss than female hearing loss, and the general view is that female hormones prevent hearing loss. The shape of the audiogram is uneven. If this is inevitable, it can only be considered that there is a congenital variation in hair cell strength during the aging process. However, the variation is individual difference and should fluctuate randomly, so it is considered that the originally obtained audiogram curves should be connected smoothly.

Non-Japanese data: Fig. 3 shows the non-Japanese averaged audiogram. There was no data below 125Hz. Similar to the Results of Japanese, the difference between female and male is not clear except in the 70s. It can be said that men in their 70s are less audible than women. The energy of sounds below 200Hz is considered to be significantly smaller than the energy of higher sounds [44], but it cannot be that HR below 125Hz is almost constant regardless of age because the curves of the 10s, 20s, and 30s are mountainous. Besides, except for those in the 70s, the high frequency makes it easier to hear. These phenomena cannot be explained by fracture mechanics. It may be considered [42] that large errors occur depending on the device and method when measuring hearing. Earphones emphasize high frequencies, so using earphones when measuring hearing may not be preferable.



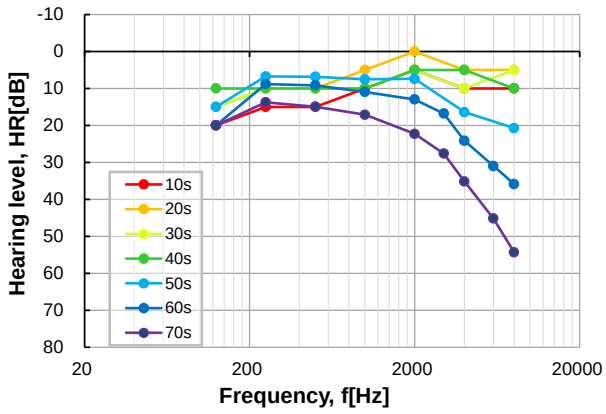
(a) Female



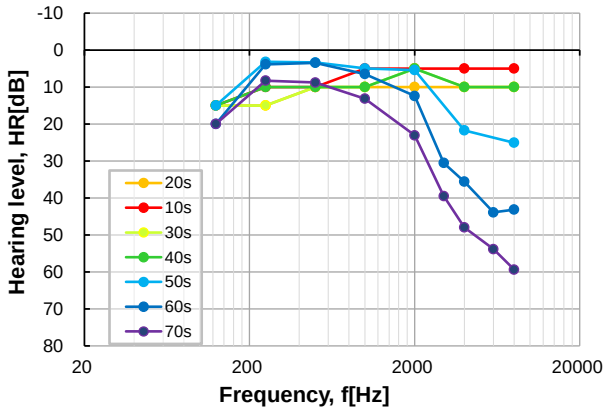
(b) Male

Fig.3 : Non-Japanese averaged audiogram.

Non-Asian data: Fig. 4 shows the non-Asian averaged audiogram. It would be inaccurate to consider the non-Asian data as Caucasian data because the data from subjects of various races may be mixed in American audiogram. Compared with Fig. 3, the hearing at 8000Hz is generally worse than at 4000Hz in Fig. 4, which can be said natural. On the other hand, the tendency for low-frequency hearing to be poor has not been resolved. The human ear becomes less sensitive at low frequencies. When the audibility is measured using sounds of the same sound pressure (volume), the low-frequency audibility appears to be poor as described above. It may be necessary to review the specific procedure for creating an audiogram. There was no data below 125Hz and extrapolation estimation cannot be done simply. Although the difference between female and male is not clear, the hearing of male tends to be worse than that of female, especially after the 50s.



(a) Female



(b) Male

Fig.4 : Caucasian averaged audiogram.

4. EAR AGE ESTIMATION ERROR

Individual Differences

Since individual differences are significantly large, error bars should be written to the averaged audiogram for the standard deviation. It is desirable to increase the number of subjects. Especially, there is too few data for frequencies above 8000Hz.

Difference Depending on Race

Compared to Japanese audiogram, non-Japanese or Caucasian one has poor low-frequency hearing. As a cause of this, for example, a difference in a device or a measurement method can

be considered. Alternatively, the hypothesis that hair cells have hardened on the contrary due to the fact that low frequencies are not used so much in everyday language holds. Racial differences are not clear and may have been hidden by other factors such as individual differences. In the future, the number of subjects should be increased for discussion.

Difference Depending on Gender

While it is inferred that there is no difference in the strength of hair cells between female and male, it is conceivable that female hair cells have high toughness due to the influence of female hormones. Assuming that male age faster than female, the frequency at which the hearing begins to drop (the curve of the audiogram begins to fall on the right side) will be different for female and male. Gender difference is not clear in Japanese data but, strictly speaking, it is considered that female and male audiograms should be used properly for ear age estimation.

Method to Predict Ear Age

The ear age estimation method used in this laboratory is as follows: The subject listens to a steady pure tone from 1kHz to 21kHz in 1kHz increments, and judges whether it is "heared", "not heard well", or "not heard at all". If the subject hears up to a certain frequency and does not hear the next higher frequency, her/his ear age is the age corresponding to the highest frequency heard. The authors have traditionally converted ear age with 40dB of P_{HL} as the borderline for deafness. In other words, the subject was considered to be 40dB audible when he/she was aware that he/she can hear. When a sound of a certain frequency was "not heard well", the median value of the ear age corresponding to the case where it was heard and that where it was not heard was adopted. The phenomenon that the subject cannot hear locally at a certain frequency suggests that she/he has partial deafness. The age corresponding to the highest frequency sound the subject can hear is her/his ear age. If he has partial hearing loss, he will be warned and advised to stop the progression of partial hearing loss by avoiding the inaudible sound source of a particular frequency.

If the boundary HR is set to 30dB instead of 40dB, the judgment of ear age will be gentler and the ear age will generally decrease. Depending on the shape of the audiogram, it is necessary to consider whether it is appropriate to estimate at HR of 40 dB, 30 dB, or 50 dB.

5. CONCLUSIONS

For the purpose of improving the estimation accuracy of ear age, audiograms were collected and error evaluation should be statistically performed in the future. Since the amount of audiograms collected this time is not sufficient, collection them is continued. In addition, different audiograms for each of the various characteristics of the subject will be drawn.

The conclusions obtained are listed below:

1) Some of the existing published audiograms do not contain subject information. There was also an audiogram made with other value as the reference sound pressure $(\Delta P)_0$. There were many audiograms that were not divided by gender.
2) When averaging, the conversion value in dB unit was statistically processed instead of the sound pressure.
3) Average audiograms for Japanese female, Japanese male, non-Japanese female, non-Japanese male, non-Asian female and non-Asian male were calculated. Non-Asian's audiograms include a mixture of subjects of came races.
4) It was suggested that the gender difference in hearing may be negligible for healthy people. On the other hand, the frequency at which the hearing begins to drop (the curve of the audiogram begins to fall on the right side) will be different for female and male.
5) Individual differences in audiograms are large.
6) The hearing survey method adopted by the authors must continue to be considered. For example, it is necessary to examine the validity of the value of 40 dB, which is the boundary line of hearing loss and which is traditionally used in our laboratory.

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Basic Research on Music Therapy
- Proposal on Timbre Comparison Experiment Method -

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ABSTRACT

Quantitative evaluation of the partial sounds of which music consists should be of importance for effective use of music. The cerebrum comprehensively recognizes the mixture of partial sounds, where the comfort of the sound is determined.

In this paper, a timbre comparison experiment is explained and the result is discussed which obtained from the experiment carried out on a group of subjects of engineering university students and music classroom students. The vague tendency is very interesting.

Many subjects are required for this experiment. While in the past, subjects were gathered into a certain place to listen to the same sound all at once to carry out the experiment, in the current situation where the new coronavirus is spreading worldwide, it is necessary to improve the method.

Keywords: Quantitative evaluation, Partial sound, Cerebrum, Hitting sound, Sound creation, Experimental survey via the internet.

1. INTRODUCTION

In conducting basic research on music therapy [1][2], it is expected that quantitative evaluation of the sounds that make up music should provide useful information when prescribing music. The sound which actually occurs is usually not a pure tone whose waveform can be mathematically expressed as a simple sine wave, and whose characteristic values are only the frequency and amplitude. Pure tones of various frequencies are mixed with each volume time change, and are recognized as one sound by the cerebrum based on the person's musical (auditory) sensibility. It can be said that there is almost no general knowledge about how to judge the comfort of sound by our cerebrum.

In our laboratory, we have been created various sounds with different partial sounds using our own sound creation program FUGA [3] and conducted experiments to compare them. In this paper, the method and the concept of the comparison experiment are explained. In addition, the experimental results of the subject group of engineering university students and that of music classroom students are carried out, and the vague tendency obtained will be explained.

2. DESIGN AND CREATION OF SOUND

Recording Original Sound

The frame was assembled and attached threads to two places in it. They hung both ends of the carbon steel plate, which is the material of the hand bell, from above to make it horizontal. In

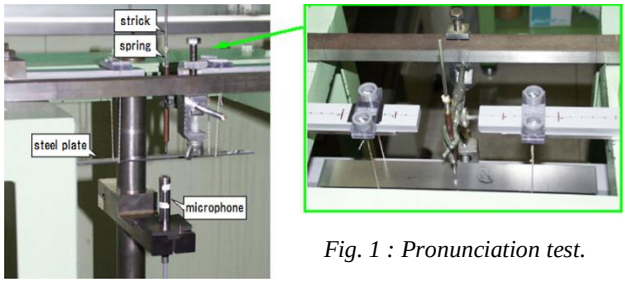
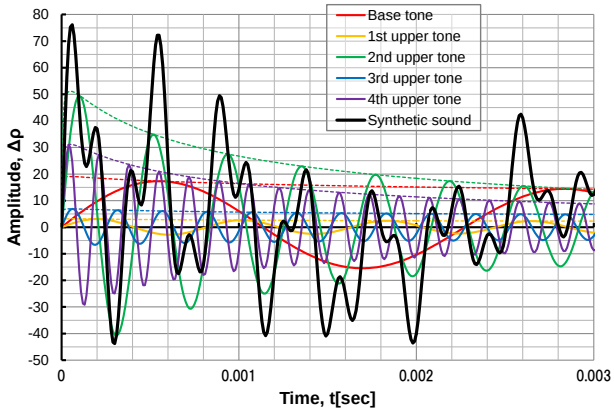


Fig. 1 : Pronunciation test.

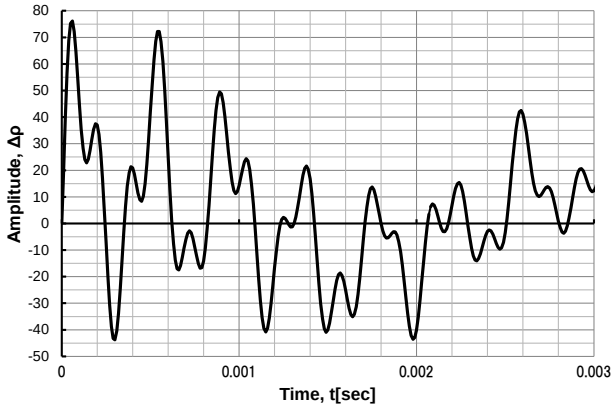
addition, the frame was equipped with a mechanism to drop the striking rod vertically, where the spring made the striking rod collided with the steel plate just only once to make a hitting sound. Then, the sound was recorded with a microphone from directly below the carbon steel plate.

Analyzing Original Sound

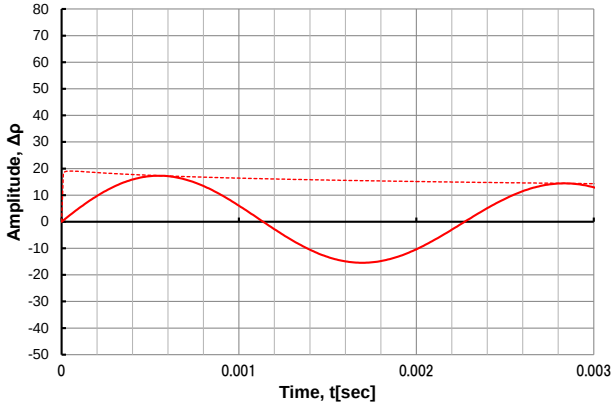
The sound was frequency analyzed and was found to consist mainly of 5 partial sounds whose waves draw the time histories as shown in Fig. 2. The base frequency was 262.5Hz, and the frequencies of each upper tone were 2.786 times, 5.452 times, 9.000 times, and 13.429 times that. The base sound, 2nd over tone and 4th over tone were rather louder. The envelopes drawn



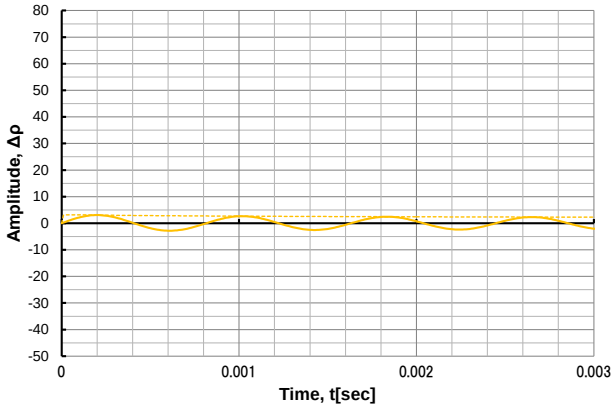
(a) Synthetic sound and all components.



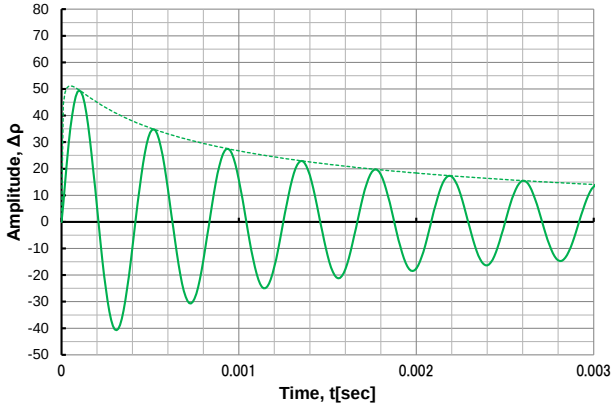
(b) Synthetic sound.



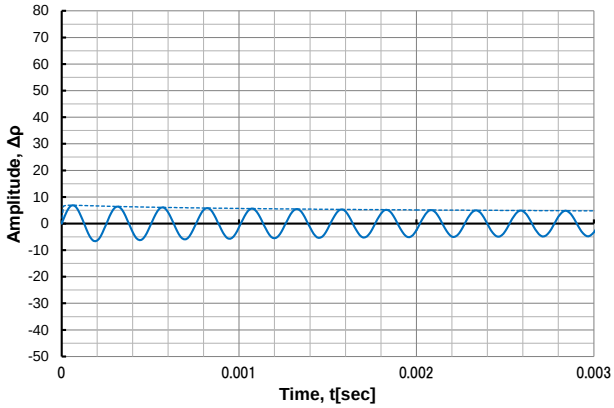
(c) Base tone.



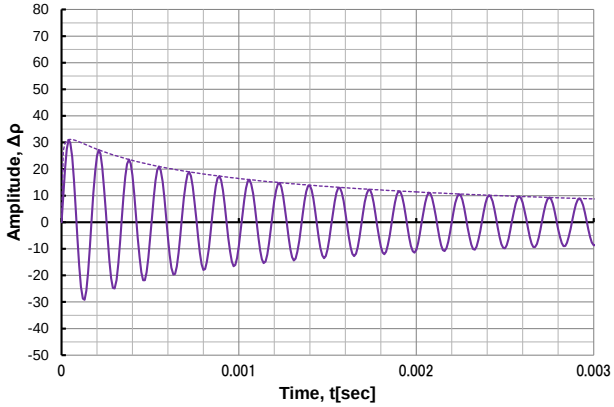
(d) 1st upper tone.



(e) 2nd upper tone.



(f) 3rd upper tone.



(g) 4th upper tone.

Fig. 2 : Time histories obtained by frequency analysis.

by the attenuation of the partial sounds were different as shown in Fig. 3, while the 2nd over tone and the 4th over tine have similar envelopes. The result suggests that the timbre of the hitting sound which generates in the actual surroundings changes subtly with the passage of time. In fact, languages are used by speaking, listening, with such timbre change.

When each envelope is expressed by Eq. (1) [3], its parameter set of each partial sound are estimated as shown in Table 1. Here, Eq. (1) can express a function that takes 0 at time 0, has a maximum value $\Delta\rho_{max}(t_{peak})$ at time t_{peak} on a smooth peak, and converges to 0, which can expectedly represent the envelope of various hitting sounds.

$$\Delta\rho_{\max}(t) = \frac{\Delta\rho_{\max}(t_{peak})}{{t_{peak}}^{m} \cdot \exp\left(-\frac{m}{n}\right)} t^{m} \cdot \exp\left(-\frac{m}{n} {t_{peak}}^{-n} t^{n}\right) \cdots (1)$$

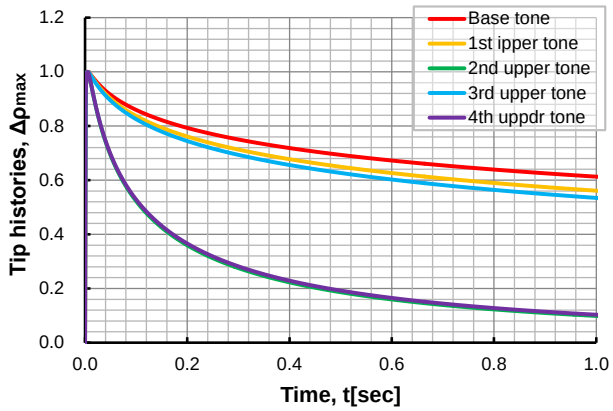


Fig. 3 : Tip histories for each component.

Table 1 : List of synthesized sounds.

			component	base	1st upper	2nd upper	3rd ipper	4th upper
Sound-A			*f*	262.5Hz	×2.786	×5.452	×9.000	×13.429
No.1 - 20		danping	t_{peak}	0.005	0.005	0.005	0.005	0.005
		parameters	*m*	0.80	0.80	0.80	0.80	0.80
		for H.S.	*n*	0.0405265	0.0472633	0.1541515	0.0508938	0.1518783
No.	type	name	factor	wright for each component				
1	H.S.	original	50	0.1908	0.0318	0.5114	0.0690	0.3115
2	H.S.	similar 1	50	0.1908	0.0000	0.5114	0.0690	0.3115
3	H.S.	similar 2	50	0.1908	0.0318	0.5114	0.0000	0.3115
4	H.S.	similar 3	50	0.1908	0.0000	0.5114	0.0000	0.3115
5	H.S.	similar 4	50	0.0000	0.0000	0.5114	0.0000	0.3115
6	H.S.	similar 5	50	0.1908	0.0000	0.0000	0.0000	0.3115
7	H.S.	similar 6	50	0.1908	0.0000	0.5114	0.0000	0.0000
8	H.S.	similar 7	50	0.3816	0.0000	0.5114	0.0000	0.3115
9	H.S.	similar 8	50	0.1908	0.0000	1.0228	0.0000	0.3115
10	H.S.	similar 9	50	0.1908	0.0000	0.5114	0.0000	0.6230
11	S.S.	original	50	0.1908	0.0318	0.5114	0.0690	0.3115
12	S.S.	similar 1	50	0.1908	0.0000	0.5114	0.0690	0.3115
13	S.S.	similar 2	50	0.1908	0.0318	0.5114	0.0000	0.3115
14	S.S.	similar 3	50	0.1908	0.0000	0.5114	0.0000	0.3115
15	S.S.	similar 4	50	0.0000	0.0000	0.5114	0.0000	0.3115
16	S.S.	similar 5	50	0.1908	0.0000	0.0000	0.0000	0.3115
17	S.S.	similar 6	50	0.1908	0.0000	0.5114	0.0000	0.0000
18	S.S.	similar 7	50	0.3816	0.0000	0.5114	0.0000	0.3115
19	S.S.	similar 8	50	0.1908	0.0000	1.0228	0.0000	0.3115
20	S.S.	similar 9	50	0.1908	0.0000	0.5114	0.0000	0.6230

H.S. for hitting sound, S.S. for steady sound

Creating Various Tones

With the parameter set, it is possible to reproduce the various sounds which are based on the original one. The tone creation program FUGA [3] created with Visual Basic outputs the sound corresponding to the input parameter set as WAVE data. The various sounds shown in Table 1 were created based on the hitting sound of the steel plate obtained as mentioned above. The sound which reproduces the original hitting sound is defined as sound 01, and the sounds in which the intensity of the component sounds of tone 01 are changed are defined as from sound 02 to sound 10. In addition, steady sounds with no attenuation and with the same timbre as that of the sounds 01 to 10 at maximum volume after hitting, have been defined as sounds 11 to 20, respectively.

3. TIMBRE COMPARISON EXPERIMENT

Original Method

While various methods can be considered as experiments to compare timbres, in our laboratory the method in which the subject listens and compares 2 sounds are employed. The reason is that it is simpler and more accurate than the method in which the subject listens multiple sounds at the same time or 3 or more sounds in sequence.

The subject listens to 2 sounds that sound for about 5 seconds in a row with an interval of about 3 seconds, where the first sound the subject hears is timbre A, and the second one is timbre B. Within approximately 10 seconds after listening to these 2 sounds, the subject replies which timbre they prefer if they have to hear again, that is, which timbre is more comfortable for them to listen to. Such listening comparisons are carried out 30 times in 1 set.

The answer sheet is shown in Fig. 4. In addition to the name and age (partially optional), the subject answers the physiological, psychological, and intellectual (sound and auditory sensibilities) conditions of the day, and then compares the timbres. To answer the comparison of timbres, the subject checks one place on the axis with 9 scales. The central scale is checked when the subject cannot determine which tone is more comfortable to hear. If the subject feels tone A to be more comfortable to hear, she/he checks by moving to the left according to the degree. If she/he feels tone B more comfortable, to the right. The subject is asked not to change through the 30 questions the criteria for determining how much she/he checks by moving to the left according to how comfortable the sound is. At the time of tabulation, the scales are assigned to -4, -3, -2, -1, 0, 1, 2, 3, and 4 from the timbre A side, and the answer contents are quantified.

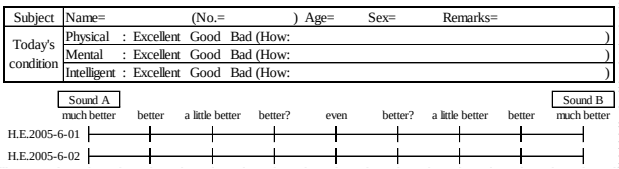


Fig. 4 : Survey sheet printed on paper.

Method Using Internet System

The original method mentioned above is realized by gathering the subjects in the same room, having them sit in front of the desk, distributing them the answer sheet, and having them listen

to the sounds through the speaker there. However, for an example, in a situation where the new coronavirus is widespread, it is not preferable for safety to gather a large number of subjects in the same room. Therefore, the authors are considering a new method using the internet and smartphone communication functions. So far, the Excel data on which the subject can hear 2 timbres and input the answer has been developed. The work sheet is shown in Fig. 5. The subject can listen to the timbres and input the answer with only this sheet. If the authors upload this Excel data to their website, subjects who are willing to answer will download it, enter it, and email them, where they can recruit a wide range of subjects. Currently, the authors are developing the websites and the smartphone applications that have similar functions.

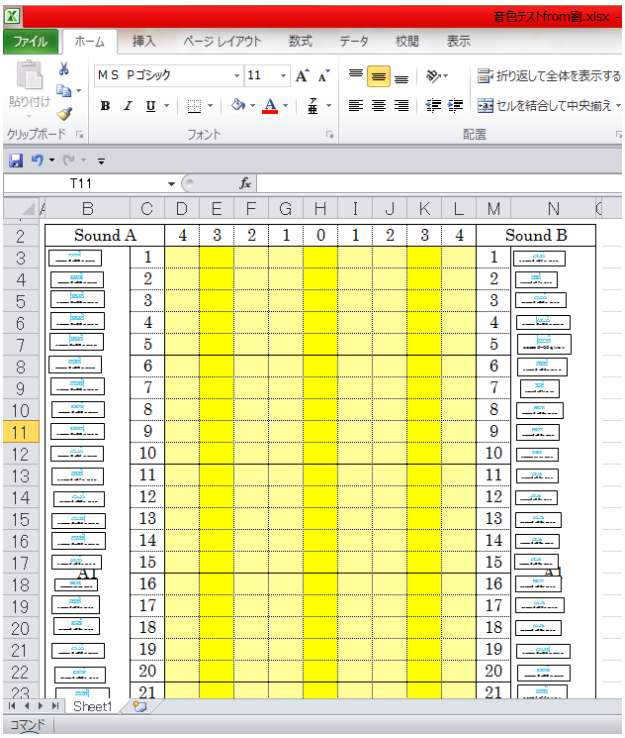


Fig. 5 : Survey sheet made in Excel data format.

Results

The results of the first experiment which were conducted in 1992 and 1993 using the original experimental method are described below. The subjects were 33 pupils learning music in the private music classroom in the city, who were 5 to 53 years old, and 12 students majoring in mechanical engineering at the graduate school. They compared timbres 01 to 10 at the 1st set of the experiment, and timbres 11 to 20 at the 2nd set. In each set, they listened to two of the 10 timbres as sound A and B, where either sound A or B was always timbre 01. That is, it was investigated how the listening comfort of other timbres 02 to 10 which have different sound components was relatively compared to timbre 01.

When quantifying the listening comfort, the numbers from -4 to 4 corresponding to the scale of the answer sheet were used. Here, the answer result is evaluated as a negative value from 0 to -4 according to the degree if the timbre 01 is more comfortable to hear, and is evaluated as a positive value from 0 to 4 if the other timbres are more comfortable. For an example, the last answer value of the subject will be 3 if the answer is 3 when she/he listens to timbre 01 as sound A and timbre 02 as sound B, and that will be 1 if the answer is -1 when she/he does timbre 02 as sound A and timbre 01 as sound B, then totally, her/his answer result will be 4, the sum of them.

Fig. 6 shows how the listening comfort of the other timbres 02 to 10 was felt compared to the timbre 01 by red marks. On the graph, the distribution of plot points shows similarities between the music classroom pupil group (solid mark) and the graduate student group (open mark), as if they were translated. Fig. 6 also shows how the listening comfort of the other timbres 12 to 20 was felt compared to the timbre 02 by blue marks. The same similarities are also seen. This similarity suggests a congenital cause that does not depend on the growth process, and our laboratory continues to study to interpret this similarity. The authors have increased the number of timbres and prepared 14 sets of timbre comparison experiments, to increase the number of data. There may hopefully be an opportunity for subsequent results to be presented in the future.

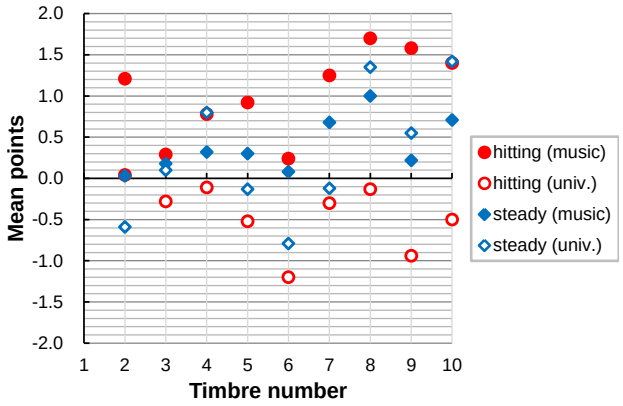


Fig. 6 : Results of timbre comparison experiments.

4. CONCLUSIONS

In parallel with basic research on music therapy, experiments to compare timbres have been conducted. In this paper, the experimental methods that we have been conducting in our laboratory was explained and the results obtained from the 1st experiment were describes. The conclusions are listed as follow:

1) In order to simplify the discussion, program FUGA, that synthesizes sound components with some parameters to create sound as WAVE data, was developed and different timbres with the different constituent partial tones parametrically were prepared.
2) As the standard timbre, the hitting sound of the steel plate which is the material of the hand bell, was adopted. The sound consisted of 5 partial sounds, 3 of which were louder than rest 2.
3) To investigate the listening comfort, the method of comparing two timbres, one of which is the comparison criteria (the reproduced timbre of the sound which actually exists) and the other of which is the artificial timbre created based on the reproduces timbre, are employed. The degree of comfort was quantified in 9 steps.
4) The groups of the pupils attending a private music classroom and that of the postgraduate students majoring in mechanical engineering participated in the first

experiment as subjects, and it was conducted on both hitting sounds and stationary sounds. There was a vague similarity between the 2 results of the both subject groups. The principle is aimed to be clarified.

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Construction of a Music Database for Earphone Hearing Loss Prevention and Music Therapy

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ABSTRACT

The basic research regarding the effects of sound and music on humans has been conducting in our laboratory. Here, it is aimed to raise awareness of the prevention of earphone hearing loss, which has become a serious issue for young people worldwide in recent years, and to establish the principle of music therapy, which is expected as a medical treatment that does not use drugs. Then, the data base of music, which is considered necessary for both of these aims, has been started building considering its concept and constitution.

This paper introduces the music database built up to this point. In addition, how to use the data base for ear age estimation in earphone hearing loss prevention research and sound effect estimation in music therapy research is proposed. In preparation for the music therapy experiment held by participating in the Beethoven 250th Anniversary Piano Recital, all of 32 Beethoven piano sonatas, 9 symphonies, and 16 string quartets have been added to the database.

Keywords: Pop Music, Rock Music, Beethoven's Symphony, Beethoven's Piano Sonatas, Beethoven's String Quartets.

1. INTRODUCTION

In recent years, earphone hearing loss (deafness) has become a serious issue worldwide, especially among young people [1][2]. In our laboratory, the basic research has been continuing with aiming to quantitatively elucidate the mechanism, to prevent and enlighten earphone hearing loss while [3][4]. In recent years, the streets have been flooded with sounds and music, and it is important to make effective use of them (music therapy, etc.) while avoiding their adverse effects. In our laboratory, the basic research has been continuing for quantitatively elucidating the mechanism of music therapy [5][6].

The authors consider that a data base of sounds and music is necessary for both of prevention of earphone hearing loss and effective use of sound and music. In our laboratory, the appropriate concept and constitution (structure) of the database have been discussed with thinking about how to apply the databases to the actual problems, and the data base has started to be constructed [7][8]. In the present paper, the music database that has been built so far is introduced. Then, the specific methods for using this database for estimating ear age in earphone hearing loss prevention research and for estimating sound effects in music therapy research is introduced.

2. BASE CONCEPTIONS

Burden on the Ears

Earphone hearing loss, which we study on, is a symptom of accelerated age-related hearing loss and is thought to be caused by the destruction of hair cells in the inner ear. That is, the root

cause of earphone hearing loss is sound energy. Therefore, the music database should have the sound energy of each piece as its element.

Hair cells are arranged in the cochlea in a finely corresponding frequency. For example, some hair cells are for listening to 1000Hz sound, and some are for listening to 10000Hz sound. Since which frequency the hair cell corresponds to depends on the resonance frequency determined by the cross-sectional shape of the basement membrane, it is considered that the shape and strength of hair cells do not differ depending on the corresponding frequency (the authors have not yet obtained information on this matter). Hair cells are damaged by the sound energy of the corresponding frequency of sound coming in from the ear. Hair cells corresponding to 1000Hz are not considered to be damaged by the sound of other frequency of sounds, for an example, 10000Hz. However, it is structurally impossible to resonate at pinpoint only at the cross-sectional part of the basement membrane that shows the natural frequency of 1000Hz when the sound of 1000Hz is input, so it is speculated that the basement membrane will resonate so that the center position becomes the swing width peak in the (small) frequency range around the corresponding frequency, such as 950Hz to 1050Hz for an example. As long as this frequency range is unknown, even if a database is created by carving frequencies into small pieces unnecessarily, there is a risk that versatility will be impaired.

In the previous report [3], the human audible range of 20Hz to 20000Hz is divided into 3 parts: a low frequency range of 20Hz to 200Hz ($10^{1.30103}=20$), a medium frequency range of 200Hz to 2000Hz, and a high frequency range of 2000Hz to 20000Hz. The frequencies were represented by 100Hz, 1000Hz, and 10000Hz for these ranges, and it was able to evaluate the ear energy to some extent. However, the ranges divided into narrower ones are necessary for a more rigorous evaluation. In this study, we compared the case where the music in the database was divided into the above three and the case where it was divided into 6 as follows: the audible frequency range is divided into 6 which are an extremely low frequency range of 20Hz to 63.25Hz ($10^{(1.30103+0.5)}=63.245554$), a low one of 63.25Hz to 200Hz, a low medium one of 200Hz to 632.5Hz, a high medium one of 632.5Hz to 2000Hz, a high one of 2000Hz to 6325Hz, and an extremely high one of 6325Hz to 20000Hz, and the frequencies of each region were represented by 50Hz, 160Hz, 500Hz, 1600Hz, 5000Hz, and 16000Hz. Here, how to divide the region is an issue that should be discussed and solved in the future.

In the calculation of sound energy, a spectrum obtained by Fourier Transforming sound data in WAVE format is required regardless of how many divisions the frequency is divided into. Therefore, the authors decided to include the spectrum analysis results as elements in the database. Based on the results of classification by the method proposed in the present study, comparing how much the sound energy varies in each group were carried out to discuss the validity of the classification.

Direction of Action on the Mind

The music therapy we study on is used for a variety of purposes. For example, it may restore a depressed feeling, or it may calm an excited feeling. It may be possible to use it for the treatment of colds in the future. Depending on the purpose, the principles and methods that music prescriptions rely on should also differ. In any case, music has a direct psychological effect rather than a physiological effect. Therefore, the authors consider that music changes the state of mind, and in this study, for the sake of simplicity, only one axis that indicates the state of mind $H(t)$, albeit vaguely are provided.

While 7 mood scales are set in POMS [9][10], it is considered that each of them is fundamentally related to how active the mind is and how the activity of the action is about to change. In this study, the state of mind is regarded as an index such as the degree of activity of the mind. The negative side of the state value means sedation ($-\infty$ means stopping biological action), positive side means excitement ($+\infty$ means runaway biological action), and the appropriate balanced position is around 0. It is considered that the state of mind $H(t)$ increases or decreases according to the energy E input from the outside and also depending on the mental activity or mental state (the latter amount of change is represented by ΔH.). Then, the state of mind $H(t)$ at time t is calculated from the state of mind $H(t-\Delta t)$ as shown in equation (1), where Δt means a small time elapsed. This hypothesis must eventually be confirmed and examined by music therapy experiments. While how the energy E input from the outside changes the state of mind may depend on the state of mind $H(t)$ at that time and how the state of mind is about to change dH/dt, those dependencies are ignored In this study. In addition, causal analysis of increase / decrease ΔH due to mental activity and mental state is a future task.

$$H(t) = H(t-\Delta t) + func.(E) + \Delta H \quad \cdots\cdots\cdots \quad (1)$$

Based on this hypothesis, the music database should have the total energy E of the music as an element. The energy E can be calculated as the sum of the sound energies for each frequency range described above.

There are many experiences in which the intonation of music is thought to influence the ups and downs of the mind. It may be that the state of the mind $H(t)$ may increase or decrease not only by the energy E but also by the fluctuation of the input energy. In this way, even if only one state axis of the mind is provided, many mechanisms of action can be considered. In order to discuss the intonation of music, time-series information of music is needed. There are many issues that should be resolved in the future.

Evaluation Value for Sound Energy

The energy flux ϕ [J/m$^2\cdot$sec] of the standing sound wave which is transmitted in the medium of density ρ [kg/m^3] at speed c [m/sec] and whose amplitude is A [m] and frequency is f [Hz] [4][10] can be calculated by Eq. (2).

$$\phi = 2\pi^2 \rho c A^2 f^2 \quad \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots \quad (2)$$

In the case of a mixed sound consisting of partial sounds i, equation (2) is expressed as equation (3).

$$\phi = \sum_i \phi_{f_i}\ ,\ \phi_{f_i} = 2\pi^2 \rho c {A_{f_i}}^2 {f_i}^2 \quad \cdots\cdots\cdots \quad (3)$$

The calculation of the energy flux of the spectrum can be done in the same manner as that of the mixed sound. While the spectrum obtained by Fourier Transforming music has a continuous distribution, there are 2 methods to calculate its total energy flux, one is to consider only large peaks and the other is to integrate on the frequency axis. It is generally said that the former has sufficient accuracy. The spectrum output by the program on the PC is digital data that consists of many partial sounds in fine frequency increments.

3. CLASSIFYING MUSIC

Essential Purpose and Policy

It is difficult to classify existing music because music has been a fusion of various fields in history. Music made by the artists of so-called Generation Z (generations born after around 2000) often does not fit into the conventional classification.

In our laboratory, the authors are constructing a music database by classifying music for the purpose of quantitatively evaluating the "burden on the ears" and the "direction of action on the mind" of the music people listen (listened) to. "Burden on the ears" is necessary information for estimating ear age, and "direction of action on the mind" is that for considering the use in music prescriptions. On the other hand, new music is constantly being supplied to the world, and it is impossible to create a database that includes all existing music. The purpose to classify music in this study is to group music that has the same (similar) "burden on the ears" and "direction of action on the mind" into the same group, and to infer which classification it is likely to belong to when you touch music that is not yet in the database.

Therefore, the viewpoint of music classification in this study should be "burden on the ears" and "direction of action on the mind". For example, considering classical music, there are quiet songs which are less burdensome to the ears, are big songs which hurt an ear, are brave songs which activate the direction of action on the mind, and are sad songs which calm down. There is a possibility that the general classification by genre cannot always be applied as it is.

Art Music

First, artistic music should be separated from the others. This is because that the composer's own strong message is often included in the creative motivation to express independent beauty, and that, as its result, the music may shake the listener's heart violently. Gagaku is regarded as an art in Japanese music, but there are also the works for the gods and the imperial family that may have little artistry and better to be considered these separately as discussed later. Classical music is typical example which is under this category in Western music. Some art music may have been composed in the name of a dedication to God, but they should be classified as art music since the purpose of this classification is the effect on the listener. For example, Beethoven's piece presented to the aristocrat who helped him financially is artistic music and is not considered to be music of the upper class.

Heavy Metal Music

While there are various opinions about the difference between rock music and rock'n'roll music, the theory that rock music is the Caucasian music, which lacks the element of African-American music [11][12] from rock'n'roll music, is considered to be the most influential. According to this theory, Elvis Aron Presley is a rock'n'roll musician, and The Beatles is a rock band. Hard rock music, which adds elements such as high-pitched shout (screaming), heavy sound pressure, and flashy guitar play to rock music, became a boom in the early 1970s. Hard rock music, where the guitar and bass guitar are electric instruments, can be said to be loud and aggressive rock music. Heavy metal music makes hard rock music even faster, heavier, and incorporates the elements of classic music. Deep Purple and Led Zeppelin are some of the bands which connected hard rock music to heavy metal music.

One of the elements of classic music incorporated into heavy metal music is the inclusion of ideas. Second, heavy metal music theory and playing methods are based on classical music. Yngwie Malmsteen, for an example, adopted the high-speed guitar playing style influenced by Paganini's violin solo and the harmonic minor scale used in Beethoven's the 8th piano sonata “Pathétique” or Chopin's etude “Revolution” in heavy metal music. The harmonic minor scale, which raises the sound of the 6th note So by a semitone, creates tension and thrills with an augmented second interval that exists in the middle of the scale. Third, the shout is an extension of the classical vocalization. Thomas Vikströmm who is a heavy metal singer and a classical tenor singer, said that he makes a lustrous voice (clear voice without faintness) and a husky voice for classical music and heavy metal music, respectively. Unlike hard rock music to get the sympathy of the general public as popular music (pops), it would be rather conceptual like classical music.

Music for God and Upper Classes

In the history of music, there is a lot of music dedicated to God and nature. Kagura (a kind of gagaku) and buddhist music are the examples of Japanese music, and religious music is the example of overseas music. The music dedicated to God will be what appeals to the feelings of respect and fear without making too much noise rather than expresses human emotions. Some Christmas songs also correspond to such music as hymns. Considering the history of mankind, each country has music for God.

It is ambiguous whether the requiem which was composed on the Bible is for God or for people. For example, Mozart's requiem expresses intense emotions with his own feeling. Some requiems should not be classified as music dedicated to God or nature.

Music that is not religious music for God is generally called

Table 1: First step of classification the data base refers to.

			Japanese music		European music		American music	
true object to give			traditional music	non traditional music	classical music	non classic music	Caucasian music	black music
God and nature			kagura		Christian music		Caucasian spirituals	African-American Spirituals
mankind	royal aristocrats or upper classes		court gagaku		secular classical music			
	beauty or thought		art gagaku		art classical music			
	ordinary person	for army	–	Japanese pop music	a part of classical music	European pop music	American pop music	
		for child	–	Japanese army music		European army music	American army music	
		for worker	Japanese murseru music	–		European mursery music		
		for folk		–				African-American work music
			Japanese folk music			European folk music	country music	jazz, blues
			soukyoku				rock and roll music	
			nagauta, johruri music			rock music		
						hard rock music		
						heavy metal music		

secular music. The classical music and heavy metal music discussed above are also secular music. Secular music is diverse. It seems necessary to classify secular music into the music about upper class, for examples the back ground music (BGM) used for upper class events, and music that expresses the life of upper class, and other music. This is because that the composer's own will is basically not included in the music, and that, as its result, the character of the music may not be as emotional as, or less emotional than the music dedicated to God. The music is treated as disposable in some case. Such music corresponds to some gagaku in Japanese music and court music in Western music.

4. DATA BASE ON CONSTRUCTIOIN

Overall Image

First, how to classify classical music and pops was considered, because it can be thought that university students and their friends and acquaintances, who would be the first experimental subjects in the future, probably listen to classical music and pop music on a regular basis. Pops is used as a general term for popular music that has been accepted by the general public at that time. As mentioned above, African-American music (blues, jazz, etc.), American Caucasian music (country, etc.), rock'n'roll music, rock music, hard rock music, heavy metal music should be subdivided rather than in the same group “pops” in this study. The authors have previously collected various pieces, and many pieces including to the classical music, hard rock, heavy metal, and other pop music were added to the data base to be mentioned the characteristics of each genre.

Table 1 shows a classification for the music dealt with in the above discussion. This classification is not yet complete. In the future, the classification will be improved and updated while the database is constructed. Database construction for art music and hard rock music, which are the groups shown in yellow in Table 1, and other pops is progressed. Table 2 lists the number of pieces in each genre (group) which have been or are being added to the database. The classified songs were first saved with WAVE data and then were searched for MIDI data. WAVE data was provided to the frequency-analysis to obtain a spectrum which the sound energy was calculated based on. MIDI data was input to the analysis program, and the distribution of the number of pitches used was output. It is presumed that the spectrum and this distribution will be relatively similar.

Classical music and Beethoven’s Pieces

Beethoven's 9 symphonies, 32 piano sonatas, and 16string quartets have been analyzed to be added to the data base, because they were compared with heavy metal, which contains a message like classical music as mentioned above, in honor of the 250th anniversary of his birth. On the other hand, Fig. 1 shows the averaged frequency analysis result of classical music that has been added to the conventional database before Beethoven’s pieces mentioned above. In Fig. 1, the product of the square of the amplitude and the frequency were calculated as energy factors, which is proportional to the amount of energy, and summarized corresponding to the case in Table 3 where the frequency region of 20Hz to 20000Hz is divided in 1, 3, and 6.

Rock Music, Hard Rock Music and Heavy Metal Music

The pops that Japanese young people prefer listening to was investigated, and rock music was separated from them. Next, the hard rock music was separated from the rock music, and

Table 2 : List of pieces classified and added to data base.

group	musical instrument organization									number of pieces
	Oc.	S.Q.	4p	P.F.	Vn.	Kb.	Gt.	K.H.	Sa.	
heavy metal			1							1
	before organizing									6
hard rock			1							1
			1			1				3
			1	1		1				1
	before organizing									12
rock			1							14
			1	1						1
			1			1				1
			1	1		1				3
			1	1				1		1
			1	1	1					1
			1		1		1			1
	before organizing									184
pops			1	1						2
(not include above music)			1		1					1
			1	1	1					1
			1	1		1			1	1
	before organizing									779
classical				1	1	(Bach)			*	1
	1			1		(Beethoven)				9
		1				(Beethoven)				16
				1		(Beethoven)				38
		1		1		(Brahms)				1
				1	1	(Brahms)			*	1
				1		(Dobussy)				18
				1	1	(Dobussy)			*	1
				1	1	(Dvorak)			*	2
	1			1		(Chopin)				1
				1		(Chopin)				49
				1	1	(Chopin)			*	2
						(Liszt)				4
		1				(Mendelssohn)				1
				1		(Mozart)				4
				1	1	(Pachelbel)			*	1
				1	1	(Rachmaninov)			*	1
	1			1		(Rachmaninov)				1
				1	1	(Ravel)			*	1
		1		1		(Schubert)				1
				1		(Schubert)				8
				1		(Schumann)				5
				1	1	(Schumann)			*	1
Animation	before organizing									16
Jazz	before organizing									16
folk				1	1	(Russian)			*	1
	No other instrument of the song specified									9

Oc. for Orchestra
S.Q. for String Quartet, Vn for Violin (Solo)
4p means standard set, E.G., E.B., D.S. and Vo.
E.G. for Electric Guitar, E.B. for Electric Bass-Guitar, Gt. For Guitar
D.S. for Drum-Set
Vo. For Vocal
P.F. for Pinao-Forte (Solo)
Kb. for Electric Keyboard
Sa. for Saxphone
* means not original but Violin arranged

then, the heavy metal music was separated from the hard rock music. The averaged frequency analysis results of these music groups are shown in different colors in Fig. 1. Also, as with classical music, the energy factor was calculated and shown in Table 3. Compared to classical music, they looked quite different in spectrum. On the other hand, pops, rock music, hard rock music and heavy metal music had almost consistent spectra. Since air check (volume adjustment) is done when creating WAVE data, music with a certain volume or more tends to have a similar spectrum. It may be that the volume (sound energy) which comes into the ear will be different when listening to these music live, and that the listener will surely choose the volume according to the music when listening with earphones.

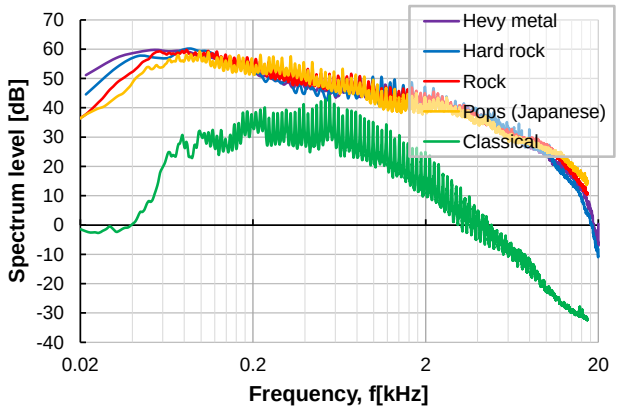

Fig. 1 : Results of frequency analyses.

Table 3 : Comparison of energy factors between music groups.

(a) Energy factors.

Division methos		Group of music				
Number	Frequency regions	Classical	Pops (Jpn.)	Rock	Hard rock	Hevy metal
1	20 - 20000Hz	2214	835630	751353	580893	657989
3	20 - 200Hz	2	8	8	8	8
	200 - 2000Hz	1160	4691	5243	5302	5086
	2000 - 20000Hz	1052	830931	746102	575582	652895
6	20 - 63.25Hz	0	0	0	0	0
	63.25 - 200Hz	2	8	8	8	8
	200 - 632.5Hz	86	200	195	172	173
	632.5 - 2000Hz	1073	4491	5048	5131	4913
	2000 - 6325Hz	1052	89587	94014	104980	100346
	6325 - 20000Hz	0	741344	652088	470602	552548

(b) Energy factor ratios to classical music.

Division methos		Group of music				
Number	Frequency regions	Classical	Pops (Jpn.)	Rock	Hard rock	Hevy metal
1	20 - 20000Hz	1	377.3	339.3	262.3	297.1
3	20 - 200Hz	1	3.2	3.4	3.3	3.2
	200 - 2000Hz	1	4.0	4.5	4.6	4.4
	2000 - 20000Hz	1	789.7	709.1	547.0	620.5
6	20 - 63.25Hz	1	16.7	19.4	15.5	17.0
	63.25 - 200Hz	1	3.1	3.3	3.2	3.2
	200 - 632.5Hz	1	2.3	2.3	2.0	2.0
	632.5 - 2000Hz	1	4.2	4.7	4.8	4.6
	2000 - 6325Hz	1	85.1	89.3	99.8	95.4
	6325 - 20000Hz	0	∞	∞	∞	∞

The energy coefficient values of 0 shown in Table 3 indicate that they are extremely close to 0. The values corresponding to the region of 20Hz – 63.25Hz are shown as 0 because the frequency is small. On the other hand, the value of the energy coefficient of classical music corresponding to the high frequency region of 6325 Hz to 20000 Hz is shown as 0. The reason is considered that classical music tends not to use high frequency sounds. Ignoring the overtones, the highest pitch of the piano, one of the highest-pitched musical instruments used to classical music, is 4186Hz. However, it would be that a good approximation result can be obtained even with 3 division method, because the shape of the spectrum is smooth, that is, there is no frequency where the energy coefficient changes rapidly.

5. CONCLUSIONS

The data base of music, which is considered necessary for researches of earphone deafness and music therapy, has been started building considering its concept and constitution. The conclusions obtained are listed as follows;

1) The classification of music is in progress. As the usage of music databases is developed and improved, the classification will be revised.
2) The data currently provided in the database are WAVE data and MIDI data which the amount of energy and the shape of the spectrum are investigated base on.
3) Classical music has a different spectrum and produces less treble than pop music.
4) It can be considered that the difference between hard rock music, including heavy metal music, and other pop music is the volume, not the shape of the spectrum.

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Experiment of Music Therapy Conducted at a Classical Music Recital - Measurement of Saliva Amylase, Hand Sweat and Muscle Hardness -

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ABSTRACT

The effect of music on the mind and body is not yet known quantitatively. In our laboratory, the basic research has been conducting for the purpose of elucidating the mechanism and referring to specific methods of music therapy. In this paper, the measurement results of salivary amylase, hand sweat, and muscle hardness performed in the music therapy experiment, which was held in a public recital. There 3 measurements suggested that Beethoven's music had a positive effect on the mind and body.

In this experiment, pulse wave, blood pressure, and cardiac directivity were also measured. The results of these measurements will be discussed in detail in another paper. Compared with these measurements, the 3 kinds of measurements described in this paper are considered to require further improvement in usage and error evaluation.

Keywords: Actual concert, Music hall, Sress and relaxation, Beethoven's piano pieces.

1. INTRODUCTION

The authors consider that the principle of music therapy has not yet been elucidated and that no specific operational method has been established for its application to real problems. In our laboratory, experiment of music therapy has been continuing. Although once every few years, experiments using an actual concert which is open to the public [2] has also be conducting. Then, subjects and experiment leaders participated in the Beethoven 250th Anniversary Recital held on December 5, 2020, and conducted music therapy experiments as the recital progressed [1].

At the experiment, 6 kinds of biometric information values were tried to be measured. In the other paper, the authors summarized the overall feeling, and reported and discussed the measurement results of pulse wave, blood pressure, and mental directivity. In the present paper, the measurement results of the remaining three types, namely salivary amylase, hand sweat, and muscle hardness will be reported and discussed. It also presents a record of detailed experimental implementation.

2. EXPERIMENTAL METHOD

Participated Recital
The specifications of the hall where the recital we attended was held are as follows;

Shape: It is a shoebox-shaped hall whose floor plan view is shown in Fig. 1. A shoe box type hall has shape of elongated one, such as rectangle or similar shape. The Großer Musikvereinssaal of the Wiener Musikverein (in Austria), Concertgebouw (in Netherlands), and The Symphony Hall in

Osaka (in Japan) are the examples.

Capasity: It has originally a capacity of 300 people, but as a part of measures to prevent infection with the new coronavirus SARS-Cov-2, the capacity was limited to 120 people on the day the experiment was conducted. It has a small foyer with mini kitchen as shown in Fig. 2.

Stage and Sound: The stage has the frontage of 14.15m and the depth of 6.4m with the echo wall with 2 doors on the right and left side. Reverberation of the entire hall is about 1.7sec. The pianist played a Steinway D-274 type piano. It may be that the reverberations overlapped excessively from the right and the left, which was probably because the number of visitors was limited.

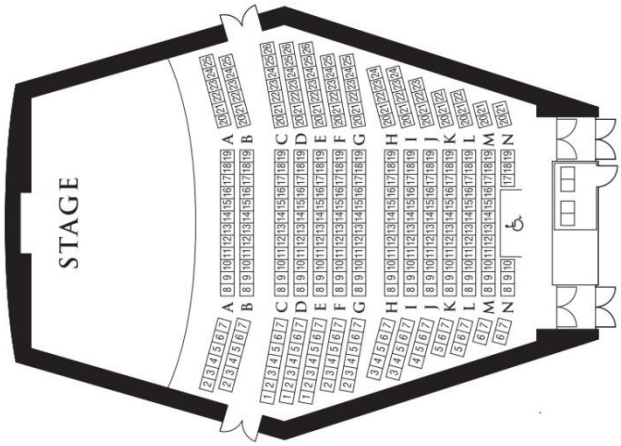

Fig. 1 : Floor plan view of hall wioth seat number [3].

Subjects

4 women and 24 men between the ages of 19 and 68 ran for the experiment as subjects. The 4 female subjects were asked to act together and therefore sit close to each other (D20, D22, E21, E23 in Fig. 1), one of whom became the experimental leader. One pulse oximeter which is a specified maintenance management medical device, one sphygmomanometer which is a home medical equipment, one saliva amylase measuring device explained below, and one muscle hardness tester explained below were lent to the 4 subjects, and the experiment leader carried out the management of measuring them.

They were classified into 4 groups according to whether they like classical music and whether they knew the pieces to be performed there. Bagatelle No. 25 WoO 59 ” Für Elise”, is a very famous piece in Japan, and Sonata No.8 Ip.13 “Grande Sonate pathétique” is not a little known. On the other hand, Sonata No. 32 Op.111, Variations Op.34, 11 New Bagatelles Op.119 are not well known to the general public in Japan. The subject who knew more than 3 pieces in the recital program, is to belong to group a/b, and one who knew 2 or less is to belong group c/d. However, the 2 subjects who participated in the experiment on the verge could not be classified. The reason is that each subject was asked to listen to the first minute of the pieces and judge whether knew or not, and that they had no time to check. The subjects belonging to groups a/b were asked to attended a lecture on the pieces in the recital program one week before the experiment, and then listen to the pieces during the week until the day of the experiment.

Fig. 2 : View of measurement at foyer before recital started.

Table 1 : List of subjects.

Characteristics			Knew or not at experiment										like or not
No.	Sex	Age	S 20	V	S 8	Elise	B 1	B 2	B 3	B 7	S 32	class	class
1	Male	28										z	z
2	Male	30	knew	knew	knew	knew	not	not	not	not	knew	a/b	a/c
3	Male	23	not	not	not	knew	not	not	not	not	not	c/d	a/c
4	Male	24										z	z
5	Male	23	knew	knew	knew	knew	knew	knew	knew	knew	knew	a/b	b/d
6	Male	23	knew	not	knew	knew	knew	knew	not	not	not	a/b	a/c
7	Female	24	knew	not	knew	not	knew	knew	not	knew	not	a/b	b/d
8	Male	21	knew	knew	knew	knew	knew	knew	knew	knew	knew	a/b	a/c
9	Male	21	not	not	knew	knew	not	not	not	not	not	c/d	a/c
10	Male	22	not	not	knew	knew	not	not	not	not	not	c/d	b/d
11	Female	21	not	not	knew	knew	knew	not	not	not	not	a/b	a/c
12	Male	21	not	not	knew	knew	not	not	not	not	not	c/d	a/c
13	Male	20	knew	knew	knew	knew	knew	not	not	not	not	a/b	a/c
14	Male	20	knew	not	knew	knew	not	knew	not	not	not	a/b	b/d
15	Male	20	not	not	not	knew	not	not	not	not	not	c/d	b/d
16	Male	23	not	not	knew	knew	not	not	not	not	not	c/d	a/c
17	Male	21	not	not	not	knew	not	not	not	not	not	c/d	b/d
18	Male	22	knew	not	not	knew	not	not	not	not	not	c/d	b/d
19	Male	21	knew	knew	knew	knew	not	not	not	not	not	a/b	b/d
20	Male	23	not	not	knew	knew	not	not	not	not	knew	a/b	a/c
21	Male	22	not	not	not	knew	not	not	not	knew	not	c/d	a/c
22	Male	23	not	not	knew	knew	knew	not	not	not	not	a/b	a/c
23	Male	22	not	not	not	knew	not	not	not	not	not	c/d	a/c
24	Male	20	not	not	not	knew	not	not	not	not	not	c/d	a/c
25	Female	19	not	not	not	knew	not	not	not	not	not	c/d	a/c
26	Male	21	knew	knew	knew	knew	not	not	not	knew	knew	a/b	a/c
27	Female	24	knew	not	not	knew	not	not	not	not	not	c/d	a/c
28	Male	68	knew	not	knew	knew	not	not	not	knew	not	a/b	a/c

S for Sonata, V for Variations, B for Bagatelle. z for classifying impossible.

Measurements

The measurements were taken with care not to disturb the general visitors of the recital. In the previous experiment carried out in an actual concert, subjects can occupy the back seat. However, they could not do this time because the hall is rather small and the number of visitors was limited. Male subjects were scattered in their favorite seats.

All subjects were measured in the foyer from 70 minutes to 30 minutes before the recital, because the staff of the recital organizer was preparing in the hall and could not enter. During

an intermission, female subjects measured at their own seats and male subjects outside the hall. Approximately 30 minutes after the recital was finished and the general visitors left the hall, the organizer allowed all subjects to take measurements at their own seats. Fig. 3 shows the view of the last measurement.

Fig. 3 : View of measurement in hall after recital finished.

3. SALIVA AMYLASE MEASUREMENT

Background

Amylase is regulated by the sympathetic nervous - adrenal medullary system [4], and several researchers [5] have hoped that a-amylase in salivary glands will be a stress marker [6]. Portable stress measuring device, COCORO METER, which is a specified maintenance management medical equipment and dry clinical chemistry analyzer for salivary amylase activity [7] is commercially available. In the experiment, all the subjects measured salivary amylase before and after the recital. They places a plastic tip coated with the reagent under the tongue and holds it for 30 seconds, and the amount of amylase is measured by inserting the chip into a measuring instrument.

Method

The female and male subjects shared 1 and 3 measuring instruments, respectively. Before inserting the measuring tip into the instrument, it is necessary to operate the tip a little. A dexterous subject can finish the operation in about 5sec, but some take over 30sec. It takes about 40sec from inserting the tip into the instrument to obtaining the result and removing the tip from the instrument. If the instrument displays "Unmeasurable", the measurement can successfully be done by restarting from the beginning in some cases, or cannot be realized due to unknown cause in other cases. The more subjects participate, the longer it takes, and the instrument makes small sound during it works. In the experiment, the authors gave up measuring during the 15-minute intermission, and the measurements were taken twice, before and after the recital.

Results

Table 2 lists the amount of amylase contained in the saliva of each subject, which was measured before and after the concert. All over, their saliva amylase decreased from 30.0 to 15.7 after the recital. 2 subjects could not measure after all. The standard deviation (S.D.) was as large as the mean, which claimed that there are large individual differences in the amount of amylase. The time that the chip was contained in the mouth and the time that the chip was taken out of the mouth and then provided to the measuring instrument differed from subject to subject. The error evaluation of this measurement method should be done. On the other hand, the result that the coefficient of variation (C.V.) after the recital was smaller than that before, suggests the sedative effect of music, which is quite interested.

The change in the amount of amylase before and after the recital is shown in Fig. 4. Overall, the amount of amylase has decreased by 14.3. S.D. of the amount of change is 37.4, which is more than 2.5 times the mean value. The change significantly depends on a subject. Subject 12 showed measured value of 193 before the recital which is because he was very nervous or excited as presumed based on the other information. Excluding him, the mean value decreased from 23.5 to 15.4.

Table 2 : Values obtained by saliva amylase measurement.

Subject No.	Measurement value		
	before	after	aft.-bef.
1	28	4	-24
2	49	13	-36
3	17	15	-2
4			
5	60	11	-49
6	4	4	0
7	38	26	-12
8	18	6	-12
9	7	4	-3
10	9	6	-3
11	44	67	23
12	193	22	-171
13	12	13	1
14	36	7	-29
15	4	14	10
16	10	20	10
17	31	12	-19
18	33	9	-24
19	7	18	11
20	16	25	9
21	33	7	-26
22	64	19	-45
23	23	22	-1
24	4	4	0
25	7	38	31
26	16	8	-8
27	17	14	-3
28			
mean	30.0	15.7	-14.3
S.D.	37.4	13.4	37.4
C.V.	1.2	0.9	-2.6

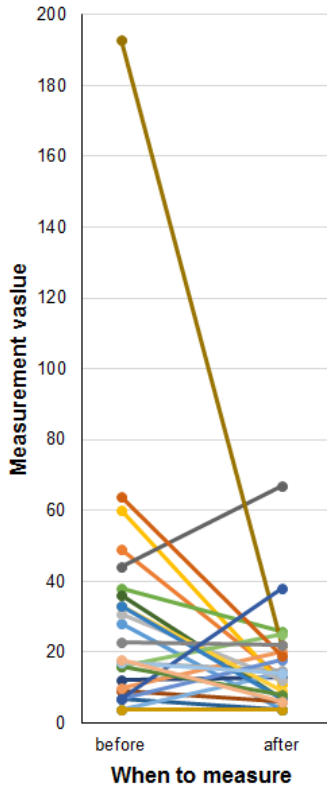

Fig. 4 : Each change of saliva amylase value.

Fig. 5 shows a histogram of the frequency distribution of the amount of change, where the class range is adopted 10. The frequency of the unchanged class (class value of 0) is the highest, and the frequency distribution is biased negatively. Considering the high possibility that stress is occurring during the measurement, the effect of music therapy was suggested.

4. HAND SWEAT MEASUREMENT

Mechanism and Method

2 types of sweat glands, eccrine glands and apocrine glands, have different sweating principles and sweat components. It is

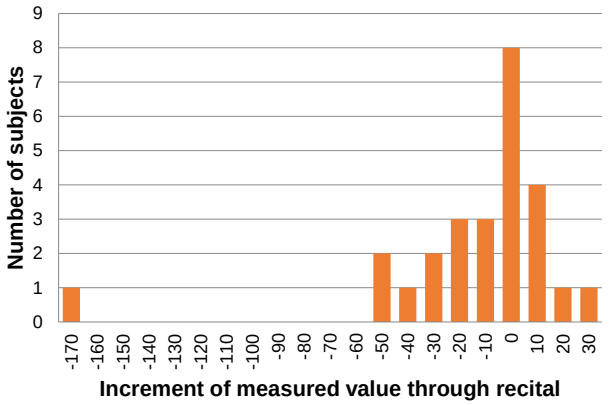

Fig. 5 : distribution of increment of saliva amylase value.

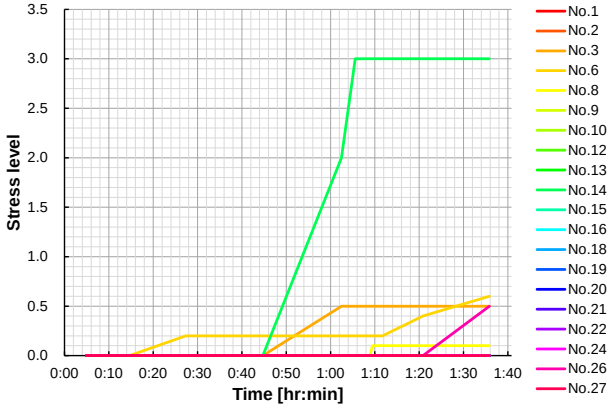

Fig. 6 : Results of stress level histories based on long term sticker.

said that the mental sweating caused by mental stimuli such as tension due to stress occurs is scratched from both glands in limited areas of the armpits, palms, and soles by neural signals from the limbic system. While there are many things which are not understand yet, there are 2 types of stickers on the market that are coated with a drug which reacts to stress-induced hand sweat. In this experiment, the required number of these 2 types of stickers was distributed to all subjects, and measurement was attempted.

Results of the Long Term Sticker

The long term sticker reacts slowly and can measure the cumulative value of stress in chronological order based on the long term sticker. Under standard stress, the amount of hand sweat is about 2mg/cm^2 per hour by the long term sheet. The concert took about 1 hour and 40 minutes, so the measurement corresponding to the standard amount of stress is about 3.6.

8 subjects couldn't obtain the results. Some of them couldn't record because they didn't have time, and some others couldn't measure because the sheet was peeled off from their hand. The other 20 subjects could record the measurement results which show the time histories as shown in Fig.6. The results of only 5 subjects showed an increase in the numerical value, and the results of the other 15 subjects remained 0 and showed the same value. At the end of the recital, the stress levels of all the subjects were below the standard value of 3.6. The stress level of Subject 14 increased from 0 to 2 during an intermission. Except for his rise during an intermission, the cumulated stress rates of the other subjects never exceeded a standard cumulative stress rate of about 2 mg/cm^2 for 1 hour. It can be said that the stress during the recital was generally less than usual.

Results of the Short Term Sticker

It reacts rapidly and can measure the cumulative value of stress received in 3 minutes. In the central circle window, this sticker displays the color which changes in response to the amount of sweating such as the continuous color from blue indicating "stressless", to red indicating "stressful". On the other hand, the continuous color pattern from blue to red is drawn around the central window. The subject can compare the color displayed on the central circle window with the color pattern to correspond the stress level to a numerical value of 1- to 4+. In this study,- and + were defined as ±0.3, respectively, and the color change was converted from 0.7 to 4.3.

The converted values obtained are shown in Table 2. The

Table 2 : Results of short term sticker.

Subject No.	Stress level at			Change of stress level between		
	before	intermission	after	int.-bef.	aft.-int.	aft.-bef.
1						
2						
3	4.0	4.0	3.3	0.0	-0.7	-0.7
4						
5	4.3	4.3	4.3	0.0	0.0	0.0
6						
7	2.0	1.0	1.0	-1.0	0.0	-1.0
8	4.0	3.7	3.3	-0.3	-0.4	-0.7
9	1.7	3.7	2.3	2.0	-1.4	0.6
10	0.7	1.7	1.7	1.0	0.0	1.0
11	3.7	2.3	2.0	-1.4	-0.3	-1.7
12						
13	2.7	2.7	3.3	0.0	0.6	0.6
14	3.7	3.7	4.3	0.0	0.6	0.6
15	1.3	3.3	3.3	2.0	0.0	2.0
16	2.3	2.3	2.7	0.0	0.4	0.4
17	1.3	3.3	3.3	2.0	0.0	2.0
18	1.0	2.0	1.0	1.0	-1.0	0.0
19						
20						
21	4.3	2.3	1.3	-2.0	-1.0	-3.0
22						
23						
24	2.3	2.3	3.0	0.0	0.7	0.7
25	2.0	2.0	2.0	0.0	0.0	0.0
26						
27	2.3	3.3	3.3	1.0	0.0	1.0
28						
mean	2.56	2.82	2.67	0.25	-0.15	0.11
S.D.	1.21	0.92	1.04	1.14	0.60	1.26
C.V.	0.47	0.33	0.39	4.53	-4.06	11.90

subjects measured with a sticker attached to the dominant hand on which they did not attach the long term sticker, before the recital started, during an intermission, and after the recital finished. 11 subjects couldn't obtain the results by the same reason as the Long Term Sticker. The difference in conversion values between each measurement showed a standard deviation (S.D.) which was significantly larger than the mean. The coefficient of variation (C.V.) means that the method for measuring the amount of sweating is unstable. In the measurement method, there are individual differences in color discrimination, and the collation of the color may be incorrect depending on the ambient light. Since the ambient light is

different at different measurement locations, it is highly possible that the three measurement conversion values cannot be compared directly. On average, the stress increased slightly after the recital compared to before it, but it is considered that the increase was not due to the effect of the performance but due to the stress caused by the task to do various other measurements [1].

5. MUSCLE HARDNESS MEASUREMENT

Background

Symptoms in the early stages of stress include stiff shoulders, low back pain, eye strain, etc. This is because the action of the sympathetic nerve causes the muscle to become tense so that it can move immediately (muscle tonus). On the contrary, it is not always the case that the degree of stress can be estimated from that of muscle tension. In addition to stress (mental state), the causes of muscle tension include posture and central nervous system diseases.

In this experiment, we measured muscle hardness as a trial and examined whether it could be a method for measuring the degree of stress. The measuring instrument used was a muscular hardness tester, TDM-NA1 [8]. Although it is not a medical device, it is lightweight and easy to operate, and it is convenient for such an experimental meeting held by participating in an actual music concert. It can be measured by pressing the stylus perpendicular to the measurement point by the measurer himself or the measurement assistant by hand.

Method

It is considered that different muscles to be measured have different muscle hardness. In addition, even for the same muscle, if the measurement points are different, the muscle hardness may be measured at different values. It is considered that subjects should not change the measurement position during the experiment (It does not refuse to measure at multiple positions.) and all subjects should measure at the same position.

It is easy to measure if the subjects have something that can be used as a mark, for an example, it is effective to put a sticker on the measurement position. On the other hand, it is difficult to specify the measurement position when wearing clothes, so it is preferable that the skin is exposed at the measurement position.

In the experiment, the muscle hardness was measured at a place where it was easy to measure from the neck to the shoulder of the trapezius muscle. 4 testers were prepared, 1 was shared by 4 female subjects and the remaining 3 were by 24 male subjects.

Results

It differs from other measurement results in that S.D. is smaller than the mean. Although there are various problems in measurement method as mentioned below, it can be said that the muscle hardness measurement is more stable than others. Overall, muscle hardness decreased from 21.88 before the recital began to 17.85 after the recital finished. The mean of the difference in muscle hardness before and after the recital is -5.37. S.D. is 4.86 and smaller than the mean, which shows listening to the Beethoven's piano pieces played in the recital can be expected to have a relaxing effect.

There was a difference between the left and right, but it can be considered that it is due to the asymmetry of the original human body and the daily activities such as how to sit. The detailed discussion will be done on the other studies.

Fig.3 : Result of muscle hardness measurement.

Subject No.	before recital			after recital			change (aft. -bef.)		
	right	left	mean	right	left	mean	right	left	mean
1	12	12	12.0	10	16	13.0	-2	4	1.0
2	9	8	8.5	12	9	10.5	4	0	2.0
3	16	20	18.0	22	13	17.5	2	-3	-0.5
4									
5	17	12	14.5	24	9	16.5	12	-8	2.0
6	20	16	18.0						
7	31	30	30.5	15	17	16.0	-15	-14	-14.5
8	31	28	29.5	20	18	19.0	-8	-13	-10.5
9	25	24	24.5	20	12	16.0	-4	-13	-8.5
10	21	21	21.0	14	15	14.5	-7	-6	-6.5
11	34	32	33.0	29	30	29.5	-3	-4	-3.5
12				17	17	17.0			
13				18	24	21.0			
14				30	37	33.5			
15				18	22	20.0			
16									
17	17	15	16.0	10	12	11.0	-5	-5	-5.0
18	24	33	28.5	23	21	22.0	-10	-3	-6.5
19	26	23	24.5	12	17	14.5	-11	-9	-10.0
20	23	33	28.0	20	16	18.0	-13	-7	-10.0
21	12	22	17.0	20	17	18.5	-2	5	1.5
22	23	21	22.0	15	14	14.5	-6	-9	-7.5
23	15	12	13.5	11	4	7.5	-1	-11	-6.0
24				20	19	19.5			
25	26	24	25.0	19	17	18.0	-5	-9	-7.0
26	26	28	27.0	24	10	17.0	-4	-16	-10.0
27	28	25	26.5	25	23	24.0	0	-5	-2.5
28									
mean	21.80	21.95	21.88	18.67	17.04	17.85	-4.11	-6.63	-5.37
S.D.	6.93	7.55	6.88	5.60	7.00	5.63	6.27	5.74	4.86
C.V.	0.32	0.34	0.31	0.30	0.41	0.32	-1.53	-0.87	-0.91

Discussions

Several subjects repeated the measurement at several positions before the experiment. The results and impressions of the subjects are described as follows:

Arm: When a measuring point is provided on the arm, they can measure by themselves at almost the same position and from the same direction with the same force each time. It is presumed that the arm is a relatively stable and easy-to-measure part, and the error is hopefully small as a result. The elbow helps the subject identify the measuring position as a guide.

Leg: If a measuring point is provided on the foot, it is more difficult to identify than that on the arm. Since the legs are below the arms, the subjects cannot measure by themselves unless they bend their body, and as a result, they will be in an unreasonable posture and it will be hard to hold the measuring position during measurement. When they wear hard clothes on their feet, it is not always possible to measure outside.

Shoulder and Back: When a measuring point is provided on the shoulder or back, it is difficult to measure multiple times at the same position because the subjects cannot see it. The measurements will be significantly different if the measuring points are slightly off, which supports that the measurement error must be large. Compared to other motor systems, various muscles are complicatedly present around the neck, shoulders, and scapula. In addition, from the shoulder to

the back, the strain of the skeleton is likely to accumulate, and the coordinate value of the measuring position may shift during the experiment. It is difficult to measure at the same position through over the clothes, because there are few information that can be used as an exact guide. The muscle hardness values measured multiple times by multiple subjects at multiple measuring points were completely unstable.

The method adopted in the experiment depends on the strength of the force to push a muscle hardness tester. In order to measure with the same force, it is necessary to prepare a measurer or practice for each person. They hinder the simplification of the experiment, but they are not impossible. There will be an error in the measurement results depending on the presence and type of clothes. Thin clothes such as T-shirts do not cause large errors, and thick clothes such as trainers and knits cause obvious errors. Women are more likely to wear underwear in layers which may result in thicker clothing and less accurate measurements. In order to confirm the effectiveness of the muscle hardness tester, it will be necessary to first conduct a laboratory- level basic study.

6. CONCLUSIONS

A music therapy experiment was conducted using an actual recital. 6 types of biometric values were measured, all of which showed that Beethoven's music had a positive effect on the mind and body. The conclusions that can be drawn from the measurements of salivary amylase, hand sweat, and muscle hardness are listed as follows:

1) **Saliva amylase**: Overall, it suggested the effect of music. There were large individual differences. Since it takes time and various measurement conditions differ depending on subjects, it is essential to improve the measurement method and evaluate the error.
2) **Hand sweat:** The long term sticker, which is available in the dark environment during the performance, suggested a slight effect of music. On the other hand, the short term sticker, which showed an unstable result with an error in color discrimination, did not conclude the musical effect. Several subjects failed to measure. Careful consideration is required for the adoption of the measurement method, and its results may be limited to reference data.
3) **Muscle harness:** The best position to measure may be on the arm. Leg is not so suitable, and it cannot be done by the subject to measure on shoulder or back. In order to measure with the same force, it is necessary to prepare a measurer or practice each person. Thick clothes such as trainers and knits cause obvious errors.

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Experiment of Music Therapy Conducted at a Classical Music Recital - Measurement of Pulse Wave, Blood Pressure and Mind Orientation -

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ABSTRACT

It is widely known that music affects a physical and mental condition, and attempts to prescribe music instead of medicine are being made in various places. However, there are large individual differences in the effects of music, and the authors consider that general prescription method has not been established yet so far.

In the present study, a music therapy experiment was conducted at an actual concert held in public. It was a piano recital commemorating the 250th anniversary of Beethoven's birth, and all the pieces played were Beethoven's. 28 subjects was women and men, aged 19 to 68, and they were classified into four groups according to whether they liked classical music and whether they knew the pieces to be performed.

Their blood pressure, pulse wave, cardiac orientation, hand sweat, salivary amylase, and muscle hardness were measured, whose results suggested that Beethoven's piano pieces generally provide a relaxing effect on the listeners. In this paper, the experimental results are discussed, which is mainly about on the pulse wave measurement conducted on 4 subjects and on the blood pressure and the directivity of the mind conducted on all subjects. Furthermore, it turned out that the method needed to be improved when experiments were conducted at an actual concert.

Keywords: Actual concert, Pulse oximeter, Profile of mood states (POMS), Stress and relaxation, Beethoven's piano pieces.

1. INTRODUCTION

It is well known that music has both of a positive and a negative effect on the physical and mental condition. Various music prescriptions are tried in clinical practice at medical sites, for an example, music therapists may be dispatched to disaster sites [1]. The authors consider that the principle of music therapy is not understood quantitatively, and that it is used in various places based on experiential knowledge.

In our laboratory, experiments of music therapy have been carried out for several years in order to pursue the principle of its effects. There are two main methods for the experiments. One is a method of conducting the experiment individually for each subject; while the location, date and time can be flexibly selected according to the convenience of each subject, the experimental conditions cannot be exactly the same, so there cannot be avoided the factors that cannot be understood. The other is a method of gathering subjects in the same concert [2]; while it is quite hard to hold the concert by yourself or to participate in an existing concert without any inconvenience, the experimental conditions are exactly the same except that they are sitting in different seats.

In this study, subjects and experimental leaders participated in an actual public recital, where they conducted a music therapy experiment as the recital progressed. The authors offered the organizer and performers of the recital in advance a desire to

conduct an experiment there, and obtained permission. The method, results and discussions are described below.

2. EXPERIMENTAL METHOD

Participated Recital

The recital we attended was Beethoven's 250th Anniversary Piano Recital which was held in the music hall in Yokohama in 5 December 2020. Its program shown in Table 1 included only Beethoven's piano pieces. It is generally said hat Beethoven's music is not suitable for music therapy because it has a strong personality and is powerful, etc., but some of the pieces played there are gentle and beautiful. It can hopefully compare whether the effects on music therapy differ depending on the pieces.

Table 1 : Time schedule of the program in the recital.

Time	Contents
0:05:00	Bell for Start
0:06:15	Sonata No.20 1st Movement
0:11:02	Sonata No.20 2nd Movement
0:14:42	Variatiuon Op.34
0:27:20	Sonata No.8 1st Movement
0:35:39	Sonata No.8 2nd Movement
0:40:19	Sonata No.8 3rd Movement
0:44:45	End of Perfprmance to Intermission
1:02:27	For Elise
1:05:35	Bagatelles No.1 Op.119-1
1:07:41	Bagatelles No.2 Op.119-2
1:08:52	Bagatelles No.3 Op.119-3
1:09:27	Bagatelles No.7 Op.119-7
1:11:43	Sonata No.32 1st Movement
1:20:50	Sonata No.32 2nd Movement
1:35:50	End of Perfprmance
1:37:34	Call of End

Subjects

28 women and men between the ages of 19 and 68 ran for the experiment as subjects. They were classified into 4 groups according to whether they like classical music and whether they knew the pieces to be performed there.

Measurements

Large-scale measurements are not possible when using an actual concert. In addition, no matter which experimental style is adopted, it is not good to attach many measuring devices to the subject. This is because the psychological effects such as discomfort and the physiological effects such as weight and pain associated with the connection of the stylus affect the mental and physical state of the subject. We can get quite a lot of information if we can measure brain waves, but it is doubtful whether that information depends solely on the effects of music.

In this experiment, the following 6 types of measurements were performed on the subject.

1) Pulse wave was measured by a pulse oximeter.
2) Blood pressure was measured by a sphygmomanometer.
3) Mind orientation was measured by POMS.
4) Saliva amylase was measured by an amylase monitor.
5) Hand sweat was measured by a measurement sticker.
6) Muscle hardness was measured by a muscle hardness tester.

3. PULSE WAVE MEASUREMENT

General Knowledge

Biometric values such as electrocardiogram (ECG), heart rate (HR) [3], brain waves and pulse waves have been measured at health examinations and clinical medicine for a long time. To estimate medical fatigue and stress [4], heart rate variability (HRV) [5], brain waves, respiratory activity, pulse waves, stress hormones, etc [6] are useful biometric information values.

The pulse wave is susceptible to the effects of stress, and can be continuously measured for a long time by a non-invasive method. The pulse wave obtained by measuring the blood flow is called blood volume pulse (BVP) [7]. BVP is analyzed directly in some case, and acceleration plethysmogram (APG) [8] obtained by differentiating BVP into the second order is analyzed in other cases. The pulse wave interval corresponds to the interval between adjacent R waves which have the largest amplitude in the electrocardiogram waveform [9], and is used as an index of parasympathetic nerve activity instead of the heartbeat. The timing does not always match the R waves [10].

HRV corresponds to the flexibility of the heart and tends to increase with younger or healthier hearts [11]. HRV is a marker of sympathetic-parasympathetic balance, as HR increases with sympathetic activity and decreases with parasympathetic. HRV is extremely small when awake or anxious, and large when relaxed and breathing slowly. HRV can be analyzed based on the peak [15] or the power spectrum density (PSD) [14] obtained by Fourier transforming the HR history. The frequency range of 0.04Hz to 0.15Hz reflects sympathetic activity [12], and that of 0.15Hz to 0.4Hz does parasympathetic [13].

Table 2 : Member list of Subjects.

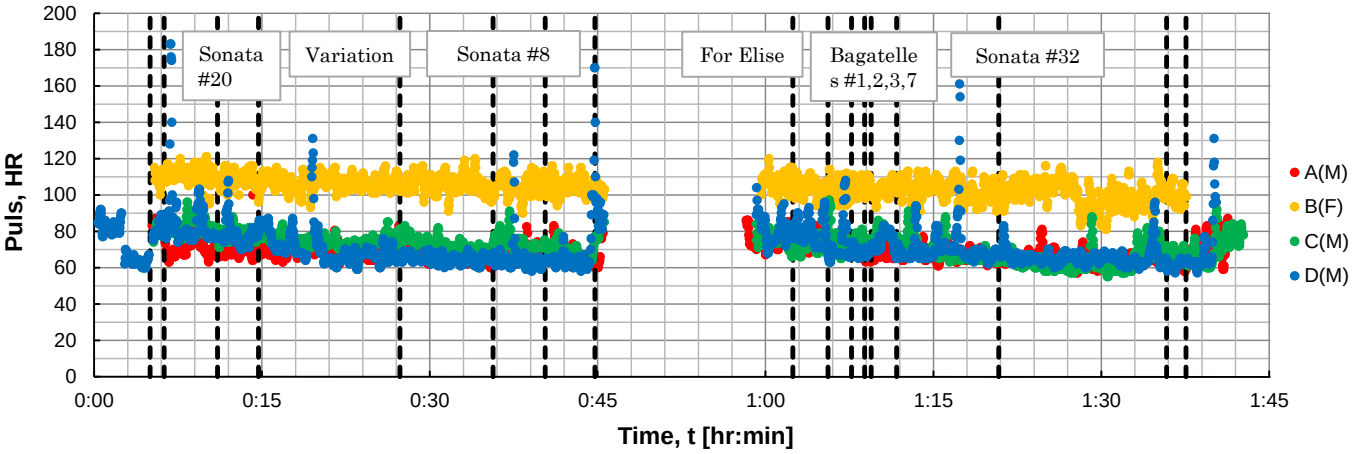
Subject	Sex	Group	classical music	pieces played in concert
A	male	c	like	had never heard
B	female	b	do not like	had heard
C	male	b	do not like	had heard
D	male	d	do not like	had never heard
non	—	a	like	had heard

Subjects

4 of the 28 subjects, referred to as subjects A, B, C, and D, cooperated in the pulse wave heart rate measurement. All were university or graduate students in their early twenties, subject B was female. Subject A belonged to group c, who likes classical music but had never heard the pieces played in the recital. Subjects B and C belonged to group b (does not but had ever), and subject D belonged to group d (does not and had never). No subject belonged to group a, who likes and had ever heard. The member list of the subjects is shown as Table 2.

Measuring Method

The changes in HR were focused. The history of pulse rate and blood oxygen saturation (SpO_2) was measured and recorded using the specified maintenance management medical device LUKRA2800 pulse oximeter. A stylus wired to the smartphone-sized main body is fitted into the index finger to measure the amount of infrared and red light transmitted through the finger along with pulse wave, and systolic and diastolic blood pressure. This device measures the degree of redness of arterial blood and calculates SpO_2. The time when the stylus is put on the finger is set as the start time, then the pulse rate and SpO_2 are recorded in the storage element in the main body every 2 seconds.



(a) Time histories of pulse HR of 4 subjects.

Each subject was seated with the pulse oximeter and operated it by him/herself to measure them. The measurement was started just before the start of the first half of the recital, was interrupted after the end of the first half, was started again just before the start of the second half, and was finished after the end of the second half. Table 1 shown above includes the track progress record of the recital. Ideally, the measurement times of all the subjects should be synchronized. However, they were at a public recital venue, so they couldn't sit next to each other or communicate with through a controller elsewhere. In addition, there may be slight variations in the speed at which the internal clock advances for each pulse oximeter. Therefore, after the experiment, the subjects were interviewed to ask when they put on and took off the stylus, what kind of subjective symptoms they had at what timing. Then, based on the information, their time axes were corrected while collating the measurement results with the progress record of the recital shown in Table 1.

Measurement of Normal Value

Before the experiment, the subjects were lent pulse oximeters so that they could read the instruction manual and make their own measurements. They measured the normal HRs to record them on the main unit, but only subject D could save his data.

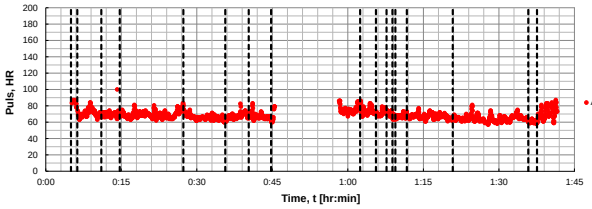
Results and Discussions

Fig. 1 shows the measurement results. The pulse histories of the 4 subjects are indicated by red, yellow, green, and blue marks. The black vertical dashed lines indicate the times of the beginning and end of the first half, the second half, and pieces played (please refer to the Table 1). The time before the earliest pre-bell corresponds before the experiment. Only subject D has pre-experimental normal data. All 4 subjects had a tendency for the pulse rate to decrease as the recital progressed. It is probable that all of them could relax by listening to the Beethoven's piano pieces and activating their parasympathetic nerves.

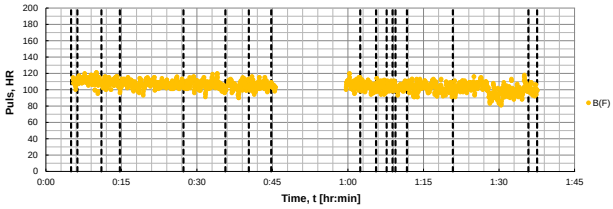
The results of each subject will be described in detail as follows.

Subject A: HR was 72 just before the start of the recital, 66 just after the end of the first half, 78 just before the start of the second half, and 62 just after the end. It declined slightly during the first half and clearly during the second half. He had a mean of 50 to 60 in normal time, and was aware that he was quite nervous at the experiment.

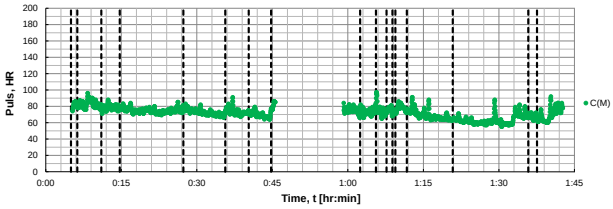
Subject B: HR was 114 just before the start of the recital, 107 just after the end of the first half, 107 just before the start of the second half, and 95 just after the end. It declined slightly during each half. She had a mean of 70 to 80 in normal time, and she was clearly nervous. She belonged to the brass band club as a high school student, and it was inferred that she was used to music. This may be one of the reasons why HR



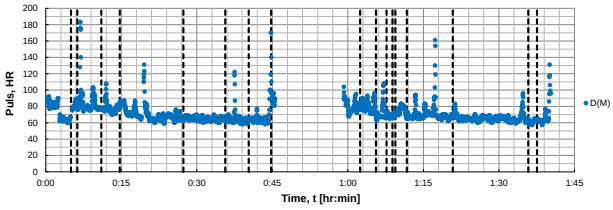
(b) Time histories of pulse HR of subject A.



(c) Time histories of pulse HR of subject B.



(d) Time histories of pulse HR of subject C.



(e) Time histories of pulse HR of subject D.

Fig 1 : Measurement Results of pulse HR.

descent was slight, and on the other hand, the HR fluctuation was constantly about 18, relatively large. She reported that she may stop breathing when listening to music. At 1:28 on the time axis, HR dropped significantly, and SpO_2 at the same time also dropped to 93%. She said she didn't like "classical" music, but the phenomenon can be thought to be due to her deep listening to the piece and a temporary decrease in breathing.

Subject C: HR was 81 just before the start of the recital, 67 just after the end of the first half, 77 just before the start of the second half, and 66 just after the end. It declined clearly during the first half and the second half. His mean HR during normal times was 70 to 75, and he was not very nervous as he realized that he was relaxed at the experiment. At 1:29 on the time axis, it rose significantly, but he had no clue.

Subject D: HR was 82 just before the start of the recital, 62 just after the end of the first half, 82 just before the start of the second half, and 62 just after the end. It declined significantly during each half. His mean in normal time is 60 to 65 according to his pre-experimental records. Even before the experiment, HR was 92. He says he has a restless and somewhat delicate, which is why it was so volatile. He reported that he was excited and that he changed his posture frequently during the experiment because of the small seat. It rose sharply just before the end of the second half, but he had no clue.

The Effect of Listening to Known Music

2 subjects in group b showed a large pulse change at about 1:30 on the time axis as mentioned above. What the pianist played then was the latter half of the 4th variation of the 2nd movement of Sonata No. 32, which was a very beautiful part of the treble. The subjects belonging to groups a and b were given a commentary on the pieces which were to be played in advance, and they were told that this part was a beautiful part. On the other hand, the other 2 subjects did not know all the pieces played there. It was suggested that if you consciously listen to the music you know, the impact (reaction) may increase.

4. BLOOD PRESSURE MEASUREMENT

Mechanism of Blood Pressure [16]

The heart is an organ that is directly affected by the balance between the sympathetic and parasympathetic nerves. Therefore, blood pressure measurement can be a convenient method for evaluating the stress that raises the sympathetic nerve.

The arterial wall is constantly exposed to internal pressure whose average is about 100mmHg, and which has fluctuations with a frequency of about 1Hz and an amplitude of about 40mmHg. Excessive hypertension [17] or hypotension causes health problems [18]. The maximum internal pressure comes out when the heart muscle contracts and tries to instantaneously send the blood that was in the left ventricle to the whole body. At the moment, about 50% of the blood in the left ventricle reaches the periphery, and the remaining blood in the aorta begins slowly pushed to the periphery as the myocardium dilates and the aortic valve closes. The internal pressure becomes the lowest [19].

Systolic blood pressure (max. pressure) is due to the ability to pump blood immediately to the periphery. It rises when cardiac function is enhanced by increased resistance due to increased blood viscosity or narrowing of blood vessels near arteries (vascular stenosis), when blood volume increases, or when the elasticity of the aorta decreases (arteries) When the heart rate rises due to stress, the systolic blood pressure rises excessively.

Diastolic blood pressure (min. pressure) occurs high when natural arterial restoration is impeded. It does, for examples, when the peripheral blood vessel is hardened, or when the peripheral blood vessels have poor blood flow due to blood clot, etc. When the systolic blood pressure rises, the diastolic blood pressure may rise accordingly, so that it may rise due to stress.

Measurement Methods

Blood pressure measurement method is classified into an indirect method (non-invasive measurement) that do not significantly damage the living body, and a direct method (invasive measurement). The indirect method measures from the body surface using a cuff or a sensor. The basic method performed by doctors is that of listening to Korotkoff sounds [20], and most existing automated sphygmomanometers employ the oscillometric method. Doppler method and the pulse wave transit time method mentioned above are also indirect methods. Water-filled method in which a catheter is inserted into a blood vessel for measurement is one of the direct methods.

Blood pressure easily fluctuates greatly due to minute internal or external disturbances. Therefore, strictly speaking, it should be measured 4 to 6 times or more [21]. There are many reasons why it rises, but if it rises only then compared to normal times, it may be due to stress. It can be measured by the subject himself with a simple home medical device. It would be rational to measure it for the purpose of estimating the stress increase or decrease during a music therapy experiment. However, blood pressure measurement by a home medical device emits a sound, and must not be performed during a performance, just before the start of a performance, or just after the end of a performance.

Presently, home medical equipment for all the subjects could not be prepared, so 4 sphygmomanometers were used to be shared. In particular, that used by 4 female subjects was entrusted to one of them. Prior to the start of the recital, all the subjects measured in the hall foyer before there came any general visitors. When measuring during an intermission, the female subjects remained seated in the hall, and the male subjects moved out to a public place with sphygmomanometers. After the recital, they measured while sitting in their own seats in the hall, waiting for the audience to disappear from their seats.

Results and Discussions

Table 3 lists the values of systolic and diastolic blood pressures measured by each subject at each timing. Here, M.V., S.D. and C.V. denote mean value, standard deviation and coefficient of variation, respectively. Because they had lots of 6 items to be measured, including blood pressure, and were running out of sphygmomanometers, some subjects could hardly measure.

The mean value of systolic blood pressure was slightly lower during an intermission than before the start and after the end than during an intermission. On the other hand, it is hard to say that individual data show a unified tendency. In some cases, blood pressure rose after the first half, in others it fell, and in many cases the trends in the both halves did not match.

The measurement results of the pulse wave and those of blood pressure for the 4 subjects were compared. HR of 3 subjects except subject B who stopped measuring immediately after the

end, showed a rapid increase after the performance. Subject 11, who is subject B, had also higher measurement value of blood pressure after the end than that of pulse wave just after the performance. It is highly possible that the task to do various measurements is a kind of stress which caused their blood pressure to rise sharply. It is inferred that the measured values during an intermission and after the end shown in Table 3 are significantly higher than during listening to the performance.

It is not preferable to use a sphygmomanometer at a concert. A concert dedicated to the experiment should be held, or an environment where as many subjects as possible can measure pulse waves should be prepared.

Table 3 : Results of blood pressure measurement.

Subjects			When they measure blood pressure						Amount of change in measured value					
			before		intermission		after		int. - bef.		aft. - int.		aft. - bef.	
No.	sex	age	max.	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.	min.
1	M	25	167	95	128	85	119	80	-39	-10	-9	-5	-48	-15
2	M	25	137	65	124	70	124	66	-13	5	0	-4	-13	1
3	M	23	133	80	138	86	138	85	5	6	0	-1	5	5
4	M	24												
5	M	23	138	84	130	66	137	74	-8	-18	7	8	-1	-10
6	M	23	118	64	132	92	122	80	14	28	-10	-12	4	16
7	F	24	113	77	103	71	113	75	-10	-6	10	4	0	-2
8	M	21	118	78	126	85	125	67	8	7	-1	-18	7	-11
9	M	21	136	75	129	77	109	76	-7	2	-20	-1	-27	1
10	M	22	134	90	110	94	129	87	-24	4	19	-7	-5	-3
11	F	21	118	86	108	80	134	105	-10	-6	26	25	16	19
12	M	21	114	78	137	79	95	69	23	1	-42	-10	-19	-9
13	M	20	121	51	92	70	109	54	-29	19	17	-16	-12	3
14	M	20	115	79	132	88	124	80	17	9	-8	-8	9	1
15	M	20			139	65	132	76			-7	11		
16	M	23	127	75			120	77					-7	2
17	M	21	124	71	130	77	116	77	6	6	-14	0	-8	6
18	M	22	114	81	116	79	116	80	2	-2	0	1	2	-1
19	M	21	112	78	104	76	121	72	-8	-2	17	-4	9	-6
20	M	23	135	60	135	78	121	65	0	18	-14	-13	-14	5
21	M	22	110	58			90	52					-20	-6
22	M	23	121	73	124	76	131	78	3	3	7	2	10	5
23	M	22	104	66	120	76	116	71	16	10	-4	-5	12	5
24	M	21			120	58	113	62			-7	4		
25	F	19	96	61	92	64	92	65	-4	3	0	1	-4	4
26	M	21	127	70	139	76	135	70	12	6	-4	-6	8	0
27	F	24	121	79	121	86	117	86	0	7	-4	0	-4	7
28	M	68												
Mean			123.0	73.9	122.0	77.3	119.2	74.2	-2.1	4.1	-1.7	-2.3	-4.2	0.7
S.D.			14.0	10.3	13.7	8.8	12.6	10.6	15.0	9.7	14.0	9.0	14.3	7.7
C.V.			0.11	0.14	0.11	0.11	0.11	0.14	-7.18	2.36	-8.18	-4.01	-3.42	10.92

5. PROFILE OF MOOD STATES CHECK

Background

Profile of Mood Stated (POMS) [22][23] is the method of numerically evaluating the temporary mood and emotional state which changes depending on the conditions and environment where the respondent is placed by answering several questions. There are currently a number of research reports using POMS in a wide range of fields from frontal lobe [24] to Zen [25]. The content of the question was translated into 42 languages, and the Japanese version of POMS was published in 1994 [26]. It has been widely used in practice sites such as medical care, workplaces, schools, for treatment of mental disorders, evaluation of exercise and relaxation effects, etc.

POMS has revised the question content in response to changes in language with the times [27]. The latest POMS has 65 questions for adults and 60 for adolescents, that simultaneously calculate 7 scales of mood such as anger-hostility (AH), confusion-bewilderment (CB), depression-dejection (DD), fatigue-inertia (FI), tension-anxiety (TA), vigor-activity (VA), Friendliness (F). It has also a version with the number of questions omitted to 30 so that you can do it in a short time. The total mood disturbance (TMD) is calculated by Eq.(1). and said to mean an estimate of the overall negative emotional states.

$$TMD = AH + CB + DD + FI + TA - VA \quad \cdots \quad (1)$$

Applying to the Present Experiment

In order to make an absolute evaluation of the mental condition, it is necessary to make a judgment by collating with various cases. On the other hand, when we focus on the changes in mood before and after the recital on the seven scales, AH, CB, DD, FI, and TA may decrease if the recital has the effect of mental and physical recovery and stress relief. In the present experiment, the time-series changes before and after the recital using a shortened version of POMS will be discussed.

The subjects were handed a questionnaire to answer it and the experiment leaders collected them before the general visitors came. They did in the same way after the recital and the general visitors went out. Since there were few desks or chairs there, the subjects were given the data in Excel format along with the questionnaire so that they could answer on their smartphones.

Results and Discussions

Overall, the values on all scales were as low as 12 or less, which confirmed that no subjects had serious mental problems. The mean value of TMD decreased by 2.4 (15%) from 15.6 to 13.2, which suggest that listening to the music performance of Beethoven's compositions reduced the negative emotional state. AH, FI, and TA also decreased by listening to the performance, and it is possible that these moods improved. Some subjects reported that they were sleepy or calm during the recital. VA has also decreased, which may be because that listening to the music or listening to it in the hall would have a sedative effect.

The frequency distribution was obtained with a class range of 3 for each scale. Fig. 2 shows the distributions of frequencies (number of subjects) vs. class values (increments of each POMS values). The peak appears at class values of 0 which means no change in POMS through the recital. The frequency distribution widened more negative, that is, many people improved their negative mood after the recital compared to before.

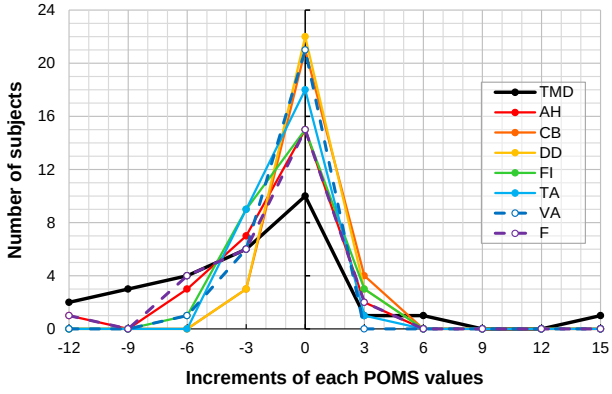


Fig.2 : Distributions of frequencies vs. class values.

The values of subject 20 are different from those of other subjects. For an example, the values other than FI showed the maximum of all subjects. He also increased the TMD frequency of class value 15. At a later date, Subject 20 confessed that he

had the next task to do after the experiment and was impatient not to be late. Subject 20 belonged to Group a (who liked classical music and knew the pieces played at the recital), and it was confirmed that the recital itself was enjoyable for him. If subject 20 is excluded, TMD will decrease by 3.0 (19%). Since the VA value indicating the active state did not change before and after the recital, the next task mentioned above would correspond to a special reason for strongly expressing a negative condition.

6. OTHER MEASUREMENTS

The measurement of saliva amylase, hand sweat, and muscle hardness cannot be completed the detailed discussion within a short page left. The authors will explain them in another presentation [32], and only the outline is described below.

Saliva Amylase Measurement
Amylase is regulated by the sympathetic nervous-adrenal medullary system [28], which can hopefully be a stress marker [29][30]. All the subjects measured using a commercially available measurement kit [31], but it is probable that the measurement conditions were different for each subject. All over, their saliva amylase decreased from 30.0 to 15.7 after the recital. However, some results showed great stress. The error evaluation of this measurement method should be done.

Hand Sweat Measurement
Two types of sticker-like sensors that can measure the amount of hand sweat were prepared for each subject. The success rate of the measurement was about 66%. Two contradictory results were obtained from the sensors which worked well; First, there was no stress increasing in many subjects through the recital. Second, the stress slightly increased in several subjects comparing before and after the recital. Both results were not obvious and require accuracy verification and error analysis.

Muscle Hardness Measurement
Test was carried out to discuss whether measuring muscle hardness could be a method to measure stress. Several subjects repeated measurement at several positions before the experiment using commercially available measuring device. All over, their muscle hardness decreased from 21.9 to 17.8 after the recital. The measurement results seem to largely depend on the presence and type of clothes. It is necessary to consider in advance which part is to be measured by whom and how.

7. CONCLUSIONS

A music therapy experiment was conducted using an actual recital, where only Beethoven's piano pieces including sonatas No.8, No.20 and No.32 were played. 28 subjects divided into four groups measured 6 types of biological information. The conclusions obtained are as follows:

1) The pulse wave measurement results supported that Beethoven's piano music had a relaxing effect. As the recital progressed, HR of all 4 subjects tended to decrease which was greater for those who knew the music.
2) POMS result and the other 4 kinds of measurement also suggest the same conclusion as what mentioned above.
3) Greater effects might be expected when a subject listens to his favorite music. As it has been considered for a long time, the authors feel that the possibility that the basis of a music prescription is to listen to the music the patient want to listen to is not low.
4) The sphygmomanometer is essentially too noisy to be used in an actual recital. While holding a recital dedicated to the experiment is one solution, it is simpler to ask each subject to measure the pulse wave.
5) In order to confirm the effectiveness of the muscle hardness measurement, it will be necessary to conduct a laboratory-level basic study.

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Exploring Prerequisites of Competence-Based Synergy in the Acquisition Processes in ICT Industry: Success Factors Approach

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This paper aims to develop and test a new research framework to assess the prerequisites of competence-based synergy in the acquisition process. To reach the aim, the research question has been pondered: How core competencies should be evaluated as sources of synergy in an acquisition? Having answered the question, I have researched the latest theoretical findings in the antecedents of synergy in the merger and acquisition process. Based on the literature review in-depth, I developed an ARCTIC framework. I selected a case study to test methodology empirically, namely, Facebook's acquisition of Instagram in 2012. The research identified three steps to explore whether core competence transfer in an acquisition process is an important source of synergy.

Keywords: synergy, core competence, M&A process, ARCTIC framework, an ICT industry

1. INTRODUCTION

The higher the level of corporate scale a firm has, the more it seeks tacit knowledge as well as codified knowledge through M&A [1]. M&A deals create more value for acquiring firm shareholders post-2009 than ever before [2]. The interrelation between different sources of relatedness in M&A transactions has been largely overlooked in extant literature [3]. Authors argue that value creation is determined by not only the target's pre-acquisition value, as indicated by numerous studies in the extant literature, but also by the acquirer's core competencies among other factors [3]. Why theoretically sound findings are usually to be lacking when subjected to empirical issues? As traditional strategic tools appear to be struggling to satisfy the need for rigor by investors, there is an increasing call for new tools and theories to evaluate new venture creation and innovations in global industries [4]. This paper aims to develop a new research framework to estimate possible competence-based synergy in the acquisition process. To reach the aim the research question has been pondered as follows: how core competencies should be evaluated as sources of synergy in merger and acquisition? The paper is organized as follows. At the beginning of the paper, I explore in-depth sources and types of synergies and the significance of the core competence concept for M&A success. There are three main sources of information that have been used in the research: business study literature, news media, and companies reports. Based on the literature review in-depth, the ARCTIC framework is developed. Then, I selected an inductive (illustrative) case study of one of the most successful Information and Communication Technology (ICT) industry giant and one of the most intriguing their M&A deals in terms of core competence transfer, specifically, successful Facebook's acquisition of Instagram in 2012 to test empirically developed ARCTIC framework. The global ICT industry was selected for the reason of global nature and major changes in its complex settings of the competitive environment. It helps to explore the competencies and knowledge transfer known as the main drivers of the successful and unsuccessful acquisition process. My research identified three steps to investigate whether core competence transfer in an acquisition process would be an important source of competence-based synergy. At the end of the paper, I discuss empirical findings, limitations, and future work. The main theoretical contribution of the research is emerging ARCTIC framework (A – Advantage, R – Relatedness, C – Complexity of Competence, T – Time of Integration, I – Implementation Plan, C – Culture compliance) for evaluation of all six critical success factors for pre-acquisition analysis of competence-based synergies.

2. LITERATURE REVIEW

Synergy in acquisition: current scientific discussion

Devers et al. [5] argue that CEOs will make acquisitions when they obtain information suggesting that the combination of their firm and a target firm offers a firm-specific synergistic opportunity to create value by exploiting one or more common or complementary resources or capabilities. The synergy effect is commonly described as 2+2=5. Rabier [6] found that reasons related to operating synergies (e.g., revenue growth through new product offerings or cost savings through economies of scale) are more likely to result in extreme high and low-performance outcomes for the acquiring firm compared to reasons related to financial synergies (e.g., diversification of cash flow streams and lowering the cost of capital). What is more, Rabier [6] found that acquisitions motivated by operating synergies have the potential to experience greater gains than acquisitions driven by financial synergies but are harder to value and implement, making them more uncertain". The study of Rozen-Bakher [7] presents a research model that includes six pre-M&A performances – the revenue and profitability of the acquirer and the target, the revenue ratio, and profitability ratio – to analyze if the pre-M&A performances could predict integration success. Thereby, much is known about the synergy in M&A, however, the knowledge is scattered and not integrated.

Success factors of an acquisition process: a resource-based view

Recently a valuable piece of the study on antecedents of M&A success was provided by Bauer and Matzler [8]. Seth [9], Homburg and Bucerius [10], King, Slotegraaf, and Kesner [11] identified and resume in their prominent research stream in strategic management literature that the strategic fit is decisive for M&A success. According to Cartwright and Schoenberg [12], Bijlsma-Frankema [13], Lodorfos and Boateng [14], Nguyen and Kleiner [15] one more important factor is cultural incompatibility or misfit, this is one of the most cited reasons for the low success rates of M&As. The post-merger integration phase is often cited to be decisive for M&A according to Haspeslagh and Jemison [16] and Stahl and Voigt [17]. The study of Angwin [18] also revealed that the speed of integration can lead to faster exploitation of synergies and faster returns on investment. Thus, synergies in an acquisition are a function of strategic similarity and complementarities which foster internal advantages and relatedness to external settings, cultural fit, and the degree and speed of integration. In absence of one or more elements, the process will go wrong from the very beginning. Having carried out the literature review, I have distinguished six critical success factors or criteria of merger and acquisition, specifically, internal advantages for both merging companies and relatedness to external settings, the complexity of technologies, cultural fit, the degree of

integration and speed of implementation. The roots of resource-based theory come from Penrose [19] whose theory of firm growth viewed organizations as bundles of resources, administered by a certain network of individuals and groups. Later studies of the 1980-s and 90-s gave way to modern resource-based theories on the firms' competitive advantages. The seminal article of Hamel and Prahalad [20] had issued a brief definition that core competence is a central value-creating capability of an organization. For these authors "core" is used to distinguish one that is fundamental to company performance and competitiveness. One more evaluation model should be mentioned, which is the most important one in terms of this study. This is Barney's [21] VRIO framework for resources, capabilities, and core competencies analysis. Barney evaluates each competence of a company in terms of its value, rareness, imitability, and organization. Although this model is initially used by the author to evaluate the company's competitive potential, Barney also outlines the potential benefits of core competence usage in M&A. The core competencies of an acquirer and a buyer should be investigated through the lens of a question if they work together? Therefore, a research question of the current paper is how core competencies should be evaluated as sources of synergy in the acquisition process? To answer a research question, the ARCTIC framework for competence-based synergies evaluation in M&A was developed.

"Every time one company launches a takeover bid for another, the justification is always about synergies… But this "sum of synergies" approach to evaluating deals is highly problematic." [22, p.2]. Dyer et al. [23] defined three different types of synergies: Sequential synergies, modular synergies, and reciprocal synergies. Authors argue that the type of synergies sought depends on the type of collaborative strategies (alliance, equity acquisition, full acquisition), nature of resources, the extent of redundant resources, the degree of market uncertainty, and the level of competition. Reciprocal synergies are when reciprocally inter-dependent resources generate synergies [23].

3. Method

Let me present the ARCTIC research framework. To construct the ARCTIC framework, I have adopted the contextual positioning method [24] by using secondary data of inductive case studies and relevant documents as extant data in grounded theory research. Contextual positioning is used as a tool that can be used to prepare extant data for analysis. "No longer is the extant data source a static collection of letters, words, sentences, and paragraphs, rather, it presents as an enlivened thing, suitably contextualized, and ready to contribute to the development of a theory grounded in data in the hands of an informed researcher [24, p 6.]". By positioning the extant data of inductive cases to study through the use of contextual positioning, I identified the key success factors of competence-based synergy and codified them in form of ARCTIC framework, locating core competencies of acquirer and target companies to appropriate factors of ARCTIC and explaining its role in the generation of competence-based synergy in M&A. Therefore, contextual positioning enhances the interactivity of the data collection process. Practically, the approach consists of three stages or steps. First, all core competencies of both companies (acquirer and target) are identified employing the VRIO framework. The second stage is a competence transferability analysis. ARCTIC framework (A – Advantage, R – Relatedness, C – Complexity of Competence, T – Time, I – Implementation Plan, C – Culture compliance) is developed to evaluate if core competencies can be transferred in the M&A process and generate synergy. The use of the model is very like four criteria (questions) of the VRIO framework, as shown in table 1. The first three criteria of the ARCTIC framework concern about potential compatibilities, similarities, and complementarily of core competencies in a new merging organization. The other three criteria are more concerned about an implementation process or an organization of core competence transfer in the M&A process. To create a competency-based synergy in the M&A process, the core competencies of both companies should satisfy all six success factors. Of course, each criterion, given as a shot question form, to be asked should be explained at length.

A – Internal Advantage – Do core competencies of one company should be further developed to promote complementarity and competence-based synergies of other companies? If the answer is "No", the competence is useless, because it does not satisfy any important needs of the acquirer's and target's companies in new core competencies, and then there is no rationale behind the merger. If the answer is "Yes", the competence is rare and difficult to imitate by several competitors, exploiting it will generate competence-based synergy in the M&A process. It is the first factor of the synergy potential of a transaction.

R- External Relatedness – Do core competencies of one company bring value that strongly depends on the environment, in which the competence will be used to other companies? If the competence is idiosyncratic and creates internal advantages, but it is not externally related to market demand, the answer is "No". If core competencies will enable companies to respond to external opportunities or threats, it is valued by customers and will underpin a new customer value proposition, the answer is "Yes". It is a second factor of the synergy potential of a transaction.

C - Complexity of core competencies – every competence has some degree of complexity, which hinders its transfer to the partners. Having core competencies that are advantageous, valuable, and related to other companies is important, but this might be not enough. If competence is based on complex technologies and sophisticated know-how, it would be difficult to transfer and grasp it by other companies. The answer to the complexity question is "No" if core competencies are complex and inappropriate to fast absorption by other company and it would hamper competence-based synergy. If the appropriation of core competencies by other companies is not so timely, costly and it is quite easy to adopt and exploit, the answer is "Yes". It is the third factor for the synergy potential of an M & A process.

T - Time of integration. Time or speed of integration can be a very important factor for successful acquisitions. It empirically tested, that the longer the integration process takes before operations start running in a normal way, the fewer chances of being successful the acquisition has. The question is as follows: do the acquisition process and post-acquisition merger are time-consuming processes or not? If valuable and rare core competence takes so much time to be transferred, that it becomes useless, the answer is "No". What is more, Rouse and Frame argue, "the sooner you select the new leaders, the quicker you can fill in the levels below them, and the faster you can fight the flight of talent and customers and the faster you can get on with the time of integration [25, p.3]". Thus, if the core competencies' transfer takes a relatively short period, the answer is "Yes". It is the fourth factor of the synergy potential of the M & A process.

I - Implementation plan – by the time top management evaluates potential acquisition, at least some steps of practical implementation should be developed. Ideally, the acquiring company should begin planning the integration process and implementation plan even before the deal is announced [25]. Does the M&A process is underpinned by detailed and coordinated planning work? If the acquirer has a focused plan that everyone understood and believed in, the answer is "Yes". When a company enters the M&A process without a strict plan to follow, again a chance of competence-based synergy is going down and the answer is "No". It is the fifth factor of the synergy potential of the M & A process.

C - Culture compliance – at last, it should be tested, how core competencies fit other company culture. "The company's leaders should consider carefully the fit with the new culture in making decisions about which people to keep [25, p. 5]". If the top

management team and staff of organizations will support and reflect the new culture, the answer is "Yes". However, if there is cultural incompatibility or misfit, the answer is "No". It is the sixth factor for the synergy potential of the M & A process. Therefore, the ARCTIC framework uses six critical success factors of competence-based synergy potential in the M&A process.

When it comes to the practical application of ARCTIC framework and verification of the judgments on all six factors, firstly, the researcher can rely on an extensive archival search that included financial statements, annual reports, internal documents, industry publications, and CEO statements of target and acquirer's companies to get at a micro-level understanding [26] that can boost the data and leads to a better understanding of the availability of the six critical success factors of competence-based synergy in M&A process.

Secondly, even though strategy-as-practice or process-based approaches in empirical qualitative research usually have an element of ethnographic or discursive analysis using primary data (sometimes in addition to secondary data, sometimes alone), the study of the ARCTIC framework's six critical factors is challenging because they are often tied to a set of variables as innovative technologies, organizational culture, etc. Regarding current research, I did not interview executives of companies due to the availability of actual interviews as secondary data sources, such as CEOs' interviews on youtube.com.

Therefore, the ARCTIC framework uses six critical success factors of competence-based synergy potential in the M&A process.

Facebook had acquired Instagram in 2012. What relevant outside knowledge, resources, and capabilities [27] has Facebook absorbed and integrated? What type of competence-based synergies has been generated by the acquisition? Or might be the cooperative arrangement (e.g., in the form of non-equity of equity alliance) would be more productive? In this vein, this acquisition has been analyzed with the application of ARCTIC and Dyer et al. frameworks within the second and third stages of the research.

The third stage of research is to justify the type of collaborative strategy (acquisition versus alliance) and to identify the type of synergies. Synergies in an acquisition are a function of strategic similarity, complementarities, and transferability of core competencies in the M&A deal. Dyer, Kale, and Singh's research [23] has made a strong contribution to correlations of the type of synergy with collaboration options. "First, companies create modular synergies when they manage resources independently and pool only the results for greater profits; the synergies are modular because modularly interdependent resources generate them. Second, firms derive sequential synergies when one company completes its tasks and passes on the results to a partner to do its bit. Third, companies generate reciprocal synergies by working closely together and executing tasks through an iterative knowledge-sharing process. For companies that desire those synergies, acquisitions are better than alliances [23, p.5]". Therefore, a competence-based synergy is a reciprocal synergy that is generated by compatibilities of partners' core competencies at collaborating [23].

4. Data Analysis and Interpretation of Results

Facebook acquisition of Instagram in 2012: absorption of mobile apps

Instagram, a popular photo-sharing app was acquired by Facebook in 2012. "Started in 2010 by Kevin Systrom and Mike Krieger, Instagram was one of the successful social media platforms with around 400 million users, globally. Instagram helped people to create an account and allowed them to make and share "artistic" photographs with their family and friends [28, p.2]". Founder and CEO Mark Zuckerberg stated on his Facebook page: "This is an important milestone for Facebook because it's the first time we've ever acquired a product and company with so many users […] But providing the best photo sharing experience is one reason why so many people love Facebook, and we knew it would be worth bringing these two companies together." To evaluate if the core competencies of Facebook and Instagram can be transferred in the acquisition process and thus generate a synergy, the ARCTIC framework is applied.

The first step is to identify the core competencies of an acquirer and a target: compatibilities, similarities, and complementarily.

According to the VRIO analysis of both companies, they have different core competencies but complement each other. Firstly, Instagram was specially designed to use on smartphones. In comparison to this, Facebook had a growing challenge to transfer its platform to a mobile application. One of the reasons for its failed success was high complexity. Due to Instagram's sustained advantages – mobile-focused application and quality and simplicity – it became possible for Facebook to successfully face this challenge. Even though Instagram had just 13 employees before the acquisition, each of them had a very special meaning for the company and its successful performance. They could come up with a variety of exceptional knowledge and important technological experiences in the ICT business, and technical start-up experience. Before the acquisition, Instagram counted over 80 million users that shared over 4 billion photos on the platform. Instagram's growing popularity was mainly based on the users' opportunity to apply stylized filters and effects on their photos. Facebook purchased the photo filter app 18 months after its launch for $1 billion.

Therefore, the strategy of buying knowledge of people of Instagram can build a reciprocal synergy (both firms execute tasks through close knowledge sharing). By combining the high frequency of photo sharing on both platforms, Facebook could raise the number of users even more. This result has been reflected in the increasing number of Instagram users' right after the acquisition.

The second step is to develop of ARCTIC framework to assess prerequisites of competence-based synergy in the acquisition process.

To test prerequisites of competence-based synergy with the application of the ARCTIC framework, I have analyzed published research articles, cases, and CEO interviews on youtube.com on that deal [29 - 32]. Facebook was born in the computers' and browsers 'era and when they acquired Instagram, the smartphones and mobile apps era was rapidly rising. Instagram was a total smartphone product, so it was a good strategic synergetic move of Facebook to strengthen their position in the global mobile ecosystem, thus, to adapt their products to future clients' needs.

Using Instagram filters and their simple design, which was completely focused on photos, Instagram could create a product that helps users to share pictures in a very easy way and transforms ordinary captures into something special and artistic. Sharing pictures was also one of the main goals of Facebook but in most cases, Facebook was not as attractive as doing it via the Instagram platform. The result of the ARCTIC analysis is presented in Table 1. According to research outcomes, both companies fulfill six critical success factors of the ARCTIC framework and, consequently, core competencies would generate a competence-based synergy emerging during and after Facebook's acquisition of Instagram. To sum up, Facebook's decision to buy Instagram seems was based on a sound strategic choice. This leads us to the conclusion that the acquisition was reasonable and provided a great synergetic strategic move of Facebook to further globally sustained success.

The third step is to identify the type of synergies and to justify the type of collaborative strategy.

Reciprocal synergies are when reciprocally inter-dependent resources generate synergies [22]. Instagram, a popular

photo-sharing app, was acquired by Facebook in 2012. Instagram was specially designed to be used on smartphones. In comparison to this, Facebook had a growing challenge to transfer its platform to a mobile application. One of the reasons for its failure was high complexity [33].

Due to Instagram's sustained advantages—a mobile-focused application and its high quality and simplicity—it became possible for Facebook to successfully face their challenge. They could come up with a variety of exceptional knowledge and important technological experiences in the ICT business. Therefore, the strategy of buying companies to obtain the knowledge of their people would build *a reciprocal synergy* involving Instagram (*both firms execute tasks through close knowledge sharing)* as shown in Table 2. By combining the high frequency of photo sharing on both platforms, Facebook could raise its number of users even more.

5. Conclusion, Research Limitations, and Future Work

My research question aimed to understand how core competencies should be evaluated as sources of synergy in acquisition/alliance strategies. The research identified three steps to investigate whether core competence transfer in an acquisition process would generate synergy. Application of the ARCTIC framework on illustrative case study shows, that the framework fulfills its purpose and helps in competence-based synergies pre-acquisition analysis, it is a main paper's contribution to theoretical and practical issues of the global M&A issues. The answers to research questions are given in tables 1 and 2. As a new research methodology, this paper highlighted the added value from the proposed ARCTIC framework which potentially would help to improve the strategic decisions in the M&A process. Six success factors of the ARCTIC framework allow to preliminarily assess core competencies as sources of potential synergy in acquisitions.

However, there are several limitations to apply of ARCTIC framework. I integrated success factors into six larger groups, for example, combining manageability and absorption capacity of technology into complexity. Decomposition of each factor of the ARCTIC framework into sub-factors (at least three in one group) will help to do new quantitative research on the ARCTIC framework. This, of course, is the perspective arena for future research.

Finally, a quite important limitation of the proposed methodology is that the ARCTIC framework assesses core competencies at the time of initiating a merger and acquisition process and evaluates only their potential to generate a competence-based synergy. The ARCTIC framework is not providing a guideline to predict how competence-based synergies would create new core competencies and what are they.

Table 1. ARCTIC framework for the evaluation of competence-based synergies (Facebook's acquisition of Instagram)

Core competence FB-Facebook IG-Instagram	Advantage? (A)	Relatedness? (R)	Lack of Complexity? (C)	Time of integration? (T)	Implementation plan? (I)	Culture compliance? (C)
FB Core competence in social platform development	Yes	Yes	Yes	Yes	Yes	Yes
FB Core competence in brand management	Yes	Yes	Yes	Yes	Yes	Yes
FB Core competence in digital technologies development	Yes	Yes	Yes	Yes	Yes	Yes
IG Core competence in photo sharing with mobile apps	Yes	Yes	Yes	Yes	Yes	Yes
IG Core competence in product development	Yes	Yes	Yes	Yes	Yes	Yes
IG Core competence in users' attraction	Yes	Yes	Yes	Yes	Yes	Yes

Source: Develop by the author.

The micro-foundations that encourage or discourage knowledge transfer [34] and foster new core competencies' creation in merging organizations are an intriguing unit of forthcoming research. What is more, cooperation and alliances play a prominent role in enhancing the ability of the firm to innovate. The ARCTIC framework might be useful to predict the synergy of cooperatives, alliances, and networking organizations. Moreover, new competence's creation in M&A, as well as knowledge leveraging, and core competencies

transformation are important dynamic capabilities [35] that would be future research papers, as in the context of already developed models, so as beyond of it.

Table 2. Identifying the type of competence-based synergy and justifying the type of collaborative strategy (Dyer et al. framework), Facebook and Instagram deal (2012).

Collaborative Strategies		
Non-equity Alliance	**Equity Alliance**	**Merger and Acquisition**
1. Type of synergy		
Modular	Sequential	Reciprocal
Modular synergies	Sequential synergies	Reciprocal synergies
		Yes
2. Nature of resources (relative value of soft to hard resources)		
Low	High	Low/Medium
Creating synergy is saddled with extensive redundant resources	Creating synergy with mostly soft resources	Creating synergy with mostly hard resources
	Yes	
3. The extent of redundant resources		
Low	Medium	High
Modular or sequential synergy using hard assets	Sequential synergy using soft assets	Reciprocal synergy using a large number of redundant resources
		Yes
4. The degree of market uncertainty		
Low	High	Low/Medium
Collaboration's synergies are highly uncertain	Collaboration's synergies are moderately uncertain	Collaboration's synergies are certain
		Yes
5. Level of competition (degree of competition for resources and users)		
Low	Medium	High
No rivals for potential partners	Some rivals for potential partners	Many rivals for potential partners
		Yes
Total		
0	**1**	**4**

Source: Develop by the author

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Venture Capital supply determinants in undeveloped markets

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ABSTRACT

A considerable amount of public funding in the form of VC has been made available in Europe, especially in its least developed parts, to decrease market gap for such funding. Still, even after many public interventions, the market's deficiencies in many countries are not eliminated, and the industry still stands on public support.

There are many studies regarding factors influencing the VC supply side and as a result ability of a particular country to have a self-sufficient VC market. The study aimed to build a comprehensive list of determinants of VC supply and identify most triggering in unmatured VC markets.

The paper presents the VC supply determinants' conceptual model. The model was built on the results of the literature content analysis. As per the model, the local market and legal environment is the most crucial determinant of the country's VC supply. The public support and the features of local society has impact on the supply, but much less than the environment and not direct but through interconnectedness with other determinants.

The validation of the conceptual model by analysing data from mature and immature VC countries confirms the validity of the proposed model. As suggested by the model, the total supply in the countries with a beneficial market and legal environment has little correlation directly from other determinants.

In the undeveloped VC countries, the market and legal environment also is the main determinant of the supply. As the environment has huge flaws there, the organic supply in these countries is very low. The public support has a stronger impact in these countries, still not sufficient in a longer period without interconnectedness with other factors substantially improve the VC supply.

Applying VC determinants' model logic it is understandable that improvement of the supply is long-term step-by-step process, which could be reached continuously providing public support and directly and indirectly improving the market and legal environment and society's acceptability and fitness for VC.

Keywords: Entrepreneurial finance, Influencing factors, Venture Capital, Supply

1. INTRODUCTION

US is acknowledged as a global leader [1], [2] of VC activity and birthplace of this kind of the investments [3]. Knowing VC investments beneficial impact on the new ventures' establishment, productivity, innovation, employment, and taxes paid [4] Europe has tried to follow US success in VC respect[5].

Over the period of 2007–2019, EU governmental agencies provided EUR 16.4 billion to VC funds [6] (calculations by the authors). In 2019 alone EUR 2.3 billion or 15.4% of the newly raised committed capital of European VC funds came from governmental agencies. Notwithstanding substantial efforts and public funding devoted to increasing VC availability in Europe, most countries are still far from self-sustainable VC industry status [2].

From European countries, the UK is an excellent example of the countries that have made progress in developing the VC industry [2], [4]. Despite having a general mature VC market, the UK acknowledges the equity gap in the regions far from the financial centres and the earliest stages of the start-ups' development [7]. It is in conformity with researchers' findings that the VC supply differs between countries [8] and also for different stages [9]. There are no countries in Europe not providing any public support for the VC industry, but its amount and relevant funding stages vary between countries. [10].

Public contributions to support European VC funds are made in two ways. Through so-called shared management interventions by each EU member state separately and by centrally managed interventions directly designed and developed by the European Commission [5]. Before and after each intervention, ex-ante and post-ante evaluations are carried out.

The existing ex-ante and post-ante evaluations mostly focus on direct assessments of the stated aims and achieved results of programmes. The development of a self-sustainable VC industry across the EU is not a direct aim of the programmes. VC is simply an instrument which may be used to achieve the goals. The direct objectives are to broaden access to finance for small and medium enterprises and to support innovation and employment.

Besides the lack of deliberate measurement of the development of the VC industry's self-sustainability the researchers [11], [12], also, pointed to two significant problems of the assessments. First, the data available for different periods and different countries are hard to compare and are not sufficient for comprehensive assessment purposes. Second, results from the programmes underway are not available early enough to implement lessons learned for the subsequent period.

In addition to the governmental assessment of public support, there is a substantial amount of the studies concerning VC supply in general. Most of these studies are based on data from the countries where VC supply is mostly provided by private actors. Researchers acknowledge that VC determinants vary on the country level [8]. Still, those countries where the VC industry does not have long history and is far from self-sustainability are weakly studied.

This study aimed to develop a comprehensive framework of VC supply determinants, taking into account differences of the countries, the interconnectedness of the factors, and a time necessary to achieve changes in these factors.

The article is organized as follows: the next section introduces with the research design. The results of the analysis are presented in the third section. Section 4 outlines the main conclusions and discussion.

2. RESEARCH DESIGN

To determine the factors influencing the VC supply, a content analysis of the literature was conducted.

Web of Science was used to find relevant studies. The search terms were: venture capital and supply. Due to the massive amount of the studies and most of them based on the previous studies' results, the search scope was limited to the latest studies (2016-2020) and studies cited in them. Web of Science search tool found 64 articles. After acquittance with them and articles cited in them, 34 articles were recognised as covering the research topic, and they were used for content analysis.

During content analysis, 28 categories were developed. An analysis of codes and categories identified in different by maturity of VC industry of the regions was also done. The selection of countries belonging to the mature VC industry was made based on the results of previous research [1], [2], [4]. As countries with mature VC industry the US, UK and Germany were counted. Theoretical studies were counted as studies from countries with mature VC industries because of the proportion of VC located in these countries [6].

The validation of the conceptual model was done with data collected by Invest Europe [6]. Invest Europe is recognised as the authoritative data source for European private equity and venture capital by institutions, including the European Commission and OECD. It collects data on more than 1,400 European VC and private equity firms.

The results of the content analysis and the conceptual model of the determinants of the VC supply proposed by the authors on the basis of the content analysis are described in the next section.

3. RESEARCH RESULTS

Twenty-eight factors influencing VC supply were identified from the studies conducted on data from mature VC markets. Thirteen from them were also found in the studies concerned unmatured markets. One category (Informal or intangible institutions) was only in the studies in countries with undeveloped VC markets. Still, additional search in other data bases identified this factor also in the studies related to mature markets [2], [13]. All factors are listed in Table 1, mentioning the frequency of their appearance in the Web of Science studies.

Table 1

The factors influencing VC supply.

No	Categories	Frequency	
		Mature markets	Undeveloped markets
1	VC firms experience	3	1
2	Number of VC firms in a market	2	
3	Investment returns	5	
4	Policy for investments in VC funds	3	
5	Foreign VC investments	2	
6	Co-investment/ Syndication possibilities	7	1
7	Proximity from core economic regions	1	
8	Domestic ecosystem	3	2
9	Technical/Research university density and student rate	2	
10	Transaction costs	2	
11	Limitations of VC funds	1	1
12	Legal environment	4	1
13	Local custom for VC	3	
14	Exit possibilities	4	1
15	Macroeconomic conditions	4	1
16	Technology innovations		1
17	Alternative investment for LPs opportunities		1
18	The number of early-stage innovative entrepreneurs seeking for VC	1	2
19	Successful entrepreneurs from prior generations	3	
20	Alternative IPO and listing regulation for SMEs	2	3
21	Possibility to get additional financing for next rounds/further grow	3	
22	Governmental policies and regulations for particular kind of investments	1	1
23	Demand for new products	3	
24	Governmental funding	5	
25	Governmental programmes encouraging investors	3	1
26	Public support for early stage	3	
27	Base of investors in VC funds	1	
28	Capital market development	2	
29	Informal or intangible institutions		1

The authors grouped all factors in three meta-factors' groups (Figure 1): i) features of the local market and legal environment; ii) local society's features; iii) public support for entrepreneurship in a particular country. All meta-factors' groups have subgroups of factors directly related to VC and those who influence all market and VC industry only as part of it.

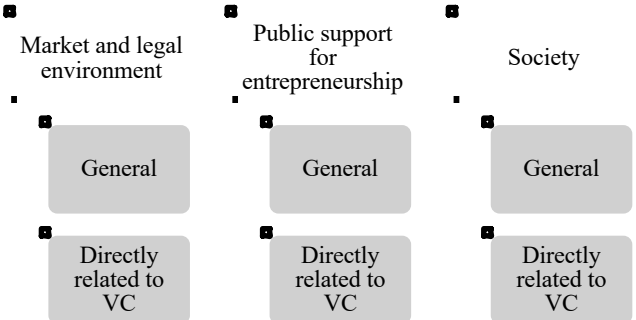

Figure 1 Meta-factors' groups of VC supply

The factors from the meta-category "Local market and legal environment" exhibit the strongest frequency of appearance – 66 (Figure 2). The frequency of factors from both other meta-categories is much lower: 12 for the meta-category "Society" and 14 for the meta-category "Public support for entrepreneurship". Similar weight of factors' groups was also observed in the studies found outside the Web of Science search.

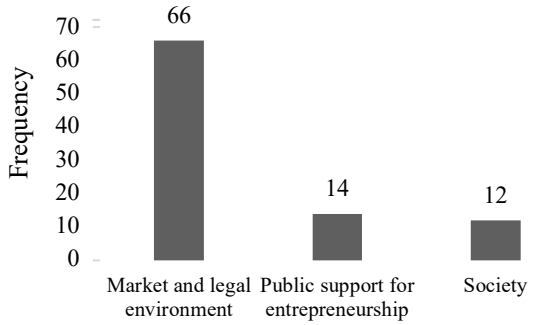

Figure 2 Frequency of the factors' appearance

Factors belonging to the meta-factors' group "Features of the local market and legal environment" are:

- General: Macroeconomic conditions, Proximity from core economic regions, Legal environment, Capital market development; Exit possibilities; Technology innovations; Alternative investment for LPs opportunities; Alternative IPO and listing regulation for SMEs; Successful entrepreneurs from prior generations; Possibility to get additional financing for next rounds/further grow.
- Directly related to VC: Number of VC firms in a market; Foreign VC investments; VC firms experience; Investment returns; Policy for investments in VC funds; Co-investment/ Syndication possibilities; Domestic ecosystem; Transaction costs.

Factors belonging to the meta-factors' group "Public support for entrepreneurship in a particular country" are:

- General: Governmental policies and regulations for particular kind of investments; Governmental programmes encouraging investors.
- Directly related to VC: Governmental funding; Public support for early stage.

Factors belonging to the meta-factors' group "Local society's features" are:

- General: Informal or intangible institutions; Technical/Research university density and student rate; Demand for new products.
- Directly related to VC: Local custom for VC; The number of early-stage innovative entrepreneurs seeking for VC.

All factors and meta-factors exhibit interdependence. Based on the content analysis results (frequency of appearance and interdependencies of factors), the authors propose a conceptual model of the VC supply determinants (Figure 3).

The content analysis allows supposing that the local market and legal environment is the primary determinant of the VC supply. If it is beneficial for VC activities, the organic growth of the supply side in a particular country is observed. If the local market and legal environment has substantial deficiencies, then the VC activity is very low, and there is no or close to no VC supply from private investors in a particular country. By providing public support it is possible to influence the supply in a particular year, but it leads to long-lasting effect only if changes in others' meta-factors groups happen organically due to increased supply or by stimulation with policy measures.

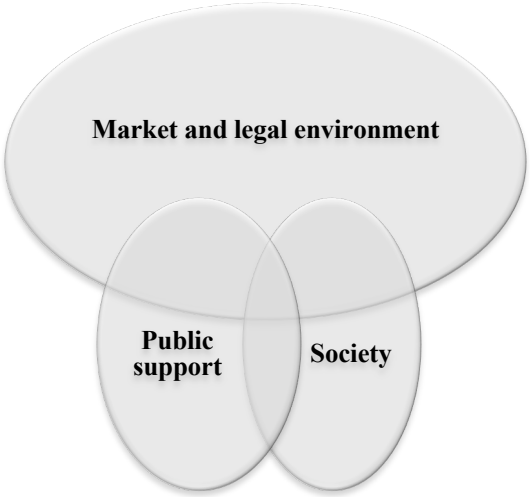

Figure 3 Conceptual model of VC supply determinants

To test the conceptual model the authors compared influence of the meta-factors' groups in UK and Baltic countries. The authors of previous studies [2], [4] have common opinion that UK has mature VC market and also has beneficial market and legal environment for that market. Baltic countries are chosen as markets with short VC industry's experience and as result unmatured market and many shortages in market and legal environment [14].

Figure 4 shows differences between VC markets in different European countries. VC activity was measured as percent of a particular country's GDP based on the data from Invest Europe [6].

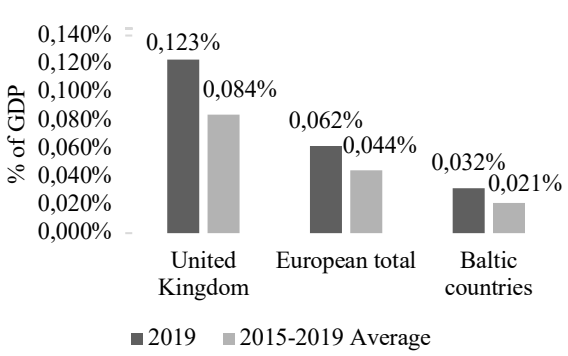

Figure 4 VC investments as percent of GDP

VC activity in UK (2015-2019) was two times higher than European average and four and mores times higher than in most undeveloped VC markets in EU, for example, in Baltic countries.

To understand the impact of the public support on VC supply, the authors measured the governmental funding level in newly raised funds (Figure 5). The data shows that even in mature markets (UK) government provides funding to support the VC supply side. Still, the percent of government funding in VC funds is substantially different between countries. During 2010-2019 UK government provided on average 12% of raised amounts. The average public support in Europe was 23 % of raised funds. But in Baltic countries average public support for VC reached 68% of totally raised funds, but in some years – even more than 90%. Also, if there were no years without public support in Europe in general, on the opposite - in Baltic countries, no public funding was provided in 2011 and 2015 and local managers could not raise any funds without the support in these years.

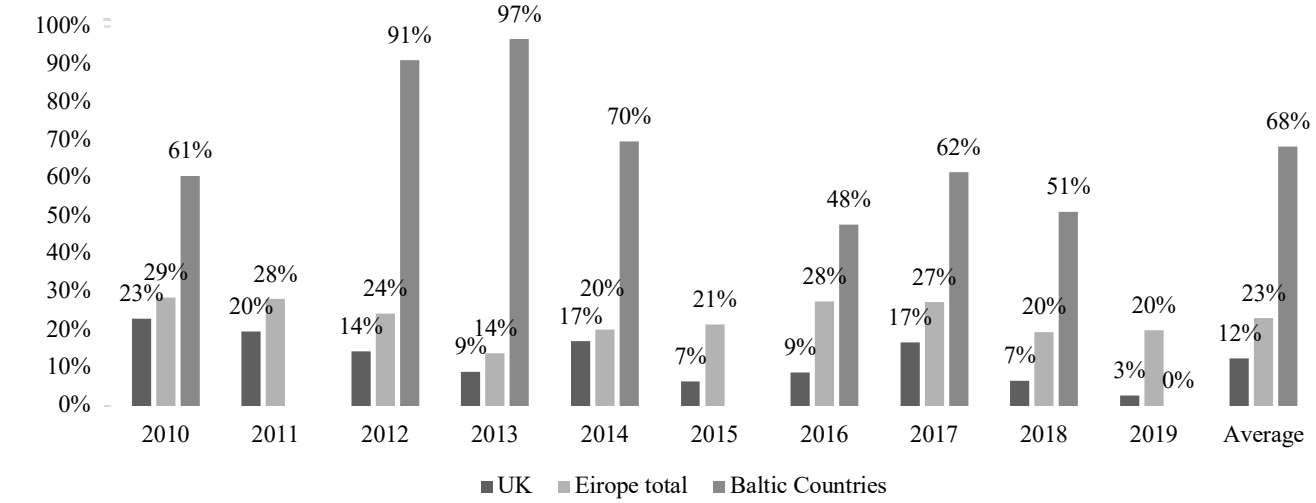

Figure 5 Percent of government funding in newly raised VC funds

Differences between UK, European average and Baltic states also appear in patterns of total amounts of newly raised funds. The total amounts of new funds increased from year to year in the UK. The same trend is observed in total European data (Figure 6).

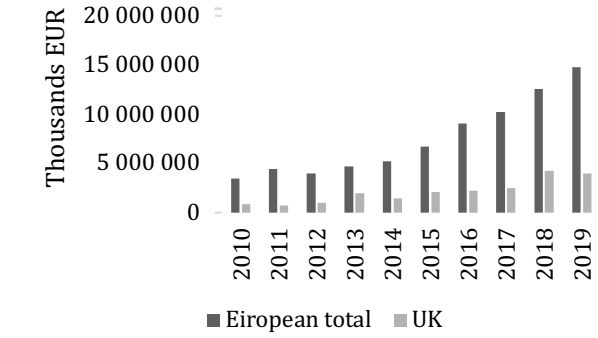

Figure 6 Funds raised during the year in UK and Europe

The pattern of the volume of new funds in Baltic countries does not exhibit the same tendency. As the amounts of total Baltic countries' funds are negligent to the European and it is impossible to see them on the same figure, the Baltic countries' volumes are provided on separate figures (Figure 7).

Data of the Baltic countries show that in a particular year public support has a strong correlation with the total VC supply in the same year. It is not a case if total European data are analysed and also particularly in the UK. Even the amount of government funding had slight fluctuations over the years, but there was no increase in the UK's average level. An increase in government funding amounts is observed in total European data, but not as a percent of newly raised funds.

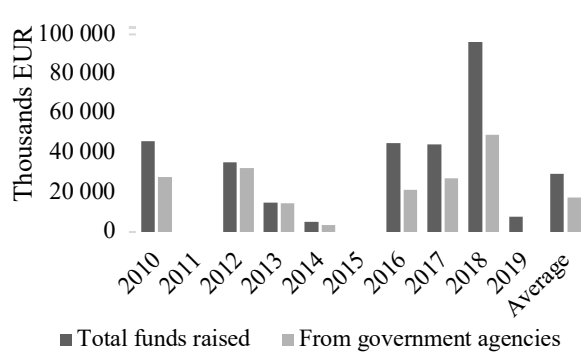

Figure 7 Funds raised in Baltic countries during the year and government investments in them

The correlation of the provided public funding in a particular year and the supply is minor in total European data and UK both in a particular year and in a longer period (2010-2019). It supports the idea of the model that public support has a little influence on VC supply in total, and the status of the supply mostly depends on the local market and legal environment.

The public support and VC total supply have a very strong correlation in a particular year in the Baltic States. In the year without public support, there was no VC supply at all. Nevertheless, when public funding is provided, it makes on average 68% of the total supply. Still, the provided public funding effect in a longer period (2010-2019) was not strong enough to generate supply by private investors in the years after public funding.

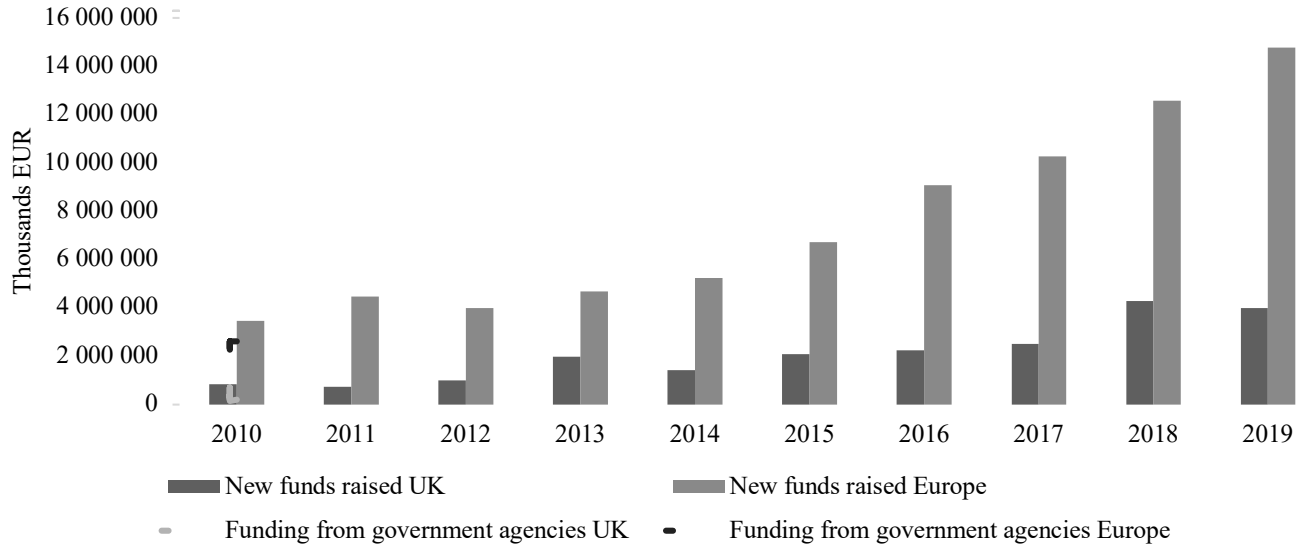

Figure 7 Funds raised in UK and Europe during the year and governmental support

However, within the next period of government support, local VC funds were able to attract more private funding. It means that the public support indirectly changes determinants of the Market and legal environment meta-group (particularly at least "Base of investors in VC funds" and "VC firms experience"). Through this indirect impact, public support increases VC supply, but very gradually.

The results suggest that public support has a much more profound impact in undeveloped markets, but it is not sufficient alone in a short period to generate the industry's ability to provide sufficient VC supply. Still, in a longer period and with influence from changes in other factors groups, it could improve VC supply in a particular country.

4. CONCLUSIONS AND DISCUSSION

The authors propose to analyse the VC supply and its public support measures through the VC supply determinants' conceptual model. The model was built on the results of the literature content analysis. As per the model, the local market and legal environment is the most important determinant of the country's VC supply. The public support and local society's features impact the supply, but much less than the environment and not direct but through interconnectedness with other determinants.

The validation of the conceptual model by analysis of data from mature and immature VC countries confirms the proposed model's validity. As suggested by the model, the total supply in the countries with the beneficial market and legal environment has little correlation with direct impact from other determinants (public support for VC particularly).

In the undeveloped VC countries, the market and legal environment also is the main determinant of the supply. As the environment has huge flaws there, the organic supply in these countries is very low. The public support has a stronger impact in these countries, still not sufficient in a longer period without interconnectedness with other factors substantially improve the VC supply. The variations in impact from different factors between developed and undeveloped VC markets also recognized in other studies [8].

It should be mentioned that during the study, the supply was analysed as a total number of the VC supply in a particular country. However, in the countries with mature VC market, public support for supply is provided not for all VC industry, but for particular stages or regions [7], [9]. Additional analysis of the VC supply model in the countries with mature markets from the perspective of particular stages and regions with funding gaps could be beneficial.

The authors suppose that an analysis of supply by stages and regions with the supply deficiencies could lead to a similar result as the result of this study regarding undeveloped markets. Particularly, in the short term, the funding gap could be eliminated by governmental support, but in a more extended period, the increase of supply would depend on public support interactions with other factors.

The public support belongs to the 3rd level of Williamson's [15] classification of institutions, and the changes in that level take effect in one to ten years. During ten years period analysed in the article (2010-2019) changes generated by the public support in the VC supply in the Baltic States are visible. Particularly, the next stage funds were able to attract more private funding. Still, this period was not sufficient to approach the self-sufficient market status. Williamson also advocates that changes in a particular institution have interconnectedness with others, and the result of changes is a sum of these changes. The proposed model is in conformity with the Williamson's theory.

The other studies conclude that public support evaluation usually happens in isolation from a long-term perspective, focusing only on a particular program [14], [16]. The model helps to understand why such an evaluation could lead to misleading results. Even with substantial public support Baltic VC industry is hardly dependant from the public support. In isolation measuring each programme, the results could lead to an opinion that the public support cannot give the desired results and therefore, it is not useful to continue it. Still, applying VC determinants' model logic it is understandable that improvement of the supply is a long-term step-by-step

process, which could be reached continuously providing public support and directly and indirectly improving the market and legal environment and society's acceptability and fitness for VC.

The model also helps to understand the existence of very different opinions regarding which market side should be boosted to increase the VC activity. There are numerous studies with results suggesting that the supply side measures are more important [16]. Other pile of the studies suggest that the market activity should be increased by boosting demand side [9]. The model's meta-category the society includes categories directly characterising the demand. As per model, the VC supply is the sum of the interconnectedness of all the meta-factors groups. Therefore, the supply is dependant from the demand. Also, the opposite is true, because the supply is generating changes in demand.

As per Williamson's theory changes in the society, its values (which is reflection and holder of a demand) happen in a long time, even in centuries [17]. A substantial shock could accelerate the pace of the changes. Regarding Baltic states, such point was the breakdown of the Soviet Union. Related to VC as accelerator can serve a local unicorn [18]. Also, economic shocks can trigger changes [19]. Deeper research of the impact of such shock factors to the VC supply would be necessary regarding undeveloped markets.

The results highlight that pressure to improve one factor without assessing its interrelation with others and its long-lasting effects may raise misleading appraisal and be a reason why ineffective policy measures are designed.

To test the robustness of the findings regarding undeveloped markets, other undeveloped markets should be analysed similarly as the Baltic States in the study. Still, previous studies also suggest that undeveloped markets have a different yield of determinants from mature markets.

5. FUNDING

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The Influence of Selected Microeconomic Factors on the Willingness of the Company Operating in Creative Industries to Collaborate

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ABSTRACT

The field of creative industries is an area that has recently become the subject of gradually increasing scientific interest. This is mainly due to how they differ compared to the more traditional sectors of the economy. We consider it necessary to examine the creative industries not only from a macroeconomic point of view or from the point of view of regional development, which are the prevailing approaches to their research, but also from a microeconomic point of view. Because only by understanding the internal logic of companies, will it be possible to understand the benefits and functionality of their internal processes. The paper aims to analyse the influence of selected factors at the company's microeconomic level on its willingness to collaborate with other companies. Because through such collaboration, it is possible to effectively share knowledge and experience in this sector of the economy, which in turn impacts companies' innovative performance. The article's research is based on a statistical analysis of the obtained data from the subsector of creative industries, namely the publishing industry. The results show a positive impact of strategic management's existence on the company's willingness to collaborate with other companies and institutions, especially with foreign companies, public institutions, and multinational companies. The influence of more liberal management approach in the company on its willingness to cooperate with its surroundings was not confirmed in the analysis. The results serve as a basis for further research in the field. Their validity must also be verified in other subsectors of the creative industries, as the creative industries cannot be considered a homogeneous sector.

Keywords: Creative Industries, Cultural Industries, Collaboration, Innovation, Strategic Management

1. INTRODUCTION

Despite its relatively long development, the creative industries remain a sector that can provoke passionate debate [1][2]. From the original negative connotation assigned by Adorno and Horkheimer to the original concept in 1944 [3], we have reached a time when the creative industries are celebrated as economic growth drivers. Today's creative industries' discourse no longer deals with their nature and significance but progresses on to discussing whether the creative industries can be considered loci of innovation and employment in a modern knowledge-oriented economy. Creative industries are directly linked to modern values, aspirations, and innovations inherent to the modern way of life, while also representing a possible future development concept [4]. The growing number of studies in the creative industries only underscores that the sector is becoming an increasingly important part of global knowledge economies [5][6].

Despite the topicality and great present value of the creative industries, the internal processes that shape and transform the creative process in the creative economy remain largely unclear or often misunderstood [7]. Thus, despite considerable progress in creative industries research, there are still areas where there is no generally accepted consensus. One of these areas is the definition of the creative industries. The main reason for the absence of a generally valid and internationally accepted definition of the creative industries is, above all, the lack of consensus on which subsectors should be considered an integral part of the creative [2].

In many, especially Western countries, current public policies often force participants in the creative industries to struggle and fight for funding of the cultural institutions they represent. Thus, there is a risk that large institutions will be much more successful in this fight than smaller ones [8]. This view only confirms the concerns of Kong [2], who see a massive threat from large multinational players, against whom small and medium-sized enterprises are losing their competitiveness.

Today, no one doubts the importance of creative industries in their innovation and growth potential for the country's economy [9]. They create space to foster human creativity, strengthen social cohesion among the population, and significantly impact societies outside the creative industries. There is evidence that companies that are not

directly part of the creative industries but are connected to them are more innovative than other companies that do not have such a connection [10]. Many authors perceive innovation as one of the key factors supporting economic growth [11]. Innovation is one of the main drivers of the creative industries, and they are among the most innovative sectors of the economy [12]. Companies in the creative industries outperform companies in other sectors regarding product innovation or research and development intensity [9].

In creative industries, work is influenced by both economic logic and internal artistic logic [13]. This fact leads to a specific organization of work in the creative industries. The organizational structure and hierarchy existing in companies operating in the creative industries are mostly informal [14]. Efforts to over-manage employees can lead to a breach of artistic integrity and logic. Therefore, traditional economic logic and standardized management practices will have a lesser chance of success in areas that fall into the creative industries [13]. Thus, the management process is highly individualized in this sector. Resource management still makes sense and is useful, but internal human resource management processes must be much more tailored to the company's specific needs than in traditional industries [15].

In most cases, companies in the creative industries are owned by individuals with a strong identity [17]. The business owner's character, identity, emotional autonomy, and good education are essential prerequisites for innovative business behaviour, even in the context of economic uncertainty, especially at the time of founding a company [16]. Innovation in companies operating in the creative industries, despite the founder's strong influence, is rarely an act of individual genius, in most cases a dynamic and collective process in which actors work complementary and mutually beneficially [6]. It is necessary to look at the business identity of the owner of the company contextually because each of the initial influences from cultural conditions, economic conditions, institutional support, to a distinct development of individual subsectors of creative industries has a significant impact on entrepreneur behaviour and psychological state [18]. This is inherent in all sectors, but we encounter a specific self-perception of entrepreneurs in the creative industries. They do not perceive themselves as entrepreneurs and are strongly reluctant to accept entrepreneurial thinking. They often feel that they must choose between cultural and creative values and entrepreneurial values. They feel that business values are at odds with their intrinsic values and consider it only a necessary evil that will enable them to realize their creative ideas. Over time, they develop a specific picture of business identity, but their creative and cultural identity remains a fundamental element of their businesses [19].

Parida et al. [20] define collaboration as the company's ability to create and use a network of current and potential inter-organizational relationships to obtain resources that other market players have and its ability to integrate parts of the organization, such as human resources partners. Collaboration is an essential prerequisite that enables establishing relationships with partners, which directly increases innovative behaviour. This creates a strong link between network capability and innovation. A surprising finding is the business owners' frequent willingness in the creative industry to collaborate, which largely contradicts a lone artists' established idea with their own truths and opinions [21].

2. CONCEPTUAL AND THEORETICAL ANALYSIS

As already mentioned in the introduction section, despite the growing notability of the creative industries and the considerable expansion of their research, their internal processes remain largely misunderstood or mostly unknown [7]. It must also be borne in mind that there is no such thing as a single creative industry because it is not a homogenous industry. Therefore, it is clear that the company's willingness to collaborate will be hidden in these internal company processes. However, as previous research suggests, entrepreneurs in the creative industries and their tendency to cooperate or compete do not depend on their age, gender, education, or income [21]. In this article's research, we will focus on microeconomic factors that could affect a company's willingness to collaborate with other companies. In particular, the relationship between the degree of strategic management in companies, which is based on whether the company sets goals and, subsequently, strategies to achieve these goals, will be analysed. As already mentioned in the introduction section, management in the creative industries due to its specific needs is possible mainly in smaller work teams because of the need for a highly individualised approach to employees. This trend is also evident in the Czech Republic, where based on the mapping of the creative industries, it was found that up to 95.9% of all entities in the creative industries were self-employed persons or micro-enterprises [22]. Another part of the analysis that we assume will impact the company's willingness to collaborate is the company's degree of liberalism. This is given by the team nature of work in the company and the degree of democracy in making company-wide decisions. Based on the mentioned assumptions, two hypotheses are formulated and subsequently verified in the article.

H1: The higher the level of the company's strategic management is, the more positive effect it has on its willingness to collaborate.

H2: The more liberal management in the company is, the more positive effect it has on its willingness to collaborate.

3. RESEARCH MEDTODOLOGY

The established hypotheses will be verified based on an analysis of data from 82 companies that completed a questionnaire. Data from these companies were obtained during the first quarter of 2020. All 82 companies belong to the publishing industry, which is a subsector of the creative industries. The paper aims to provide information on which factors may affect a company's willingness to collaborate with other companies. In addition to information on whether the company collaborates with other companies, additional information in the form of whom the company collaborate with will be used for further analysis. In the questionnaire, companies could provide information on whether they cooperate with domestic companies, foreign companies, public institutions, or multinational companies. Given the results of empirical research based on a search of the literature, the following selected microeconomic variables existing within the company will be subjected to statistical analysis:

- I_1*: Previous work experience of the business owner (executive manager) in the field of business*
- I_2*: Highest achieved education of the business owner (executive manager)*
- I_3*: Number of employees in the enterprise*
- I_4*: Gender composition of the working team*
- I_5*: Existence of goals that the company wants to achieve*
- I_6*: Existence of strategies and plans in the company*
- I_7*: Existence of an employee whose skills and experience are irreplaceable for the company*
- I_8*: Prevalence of teamwork in the company*
- I_9*: Democracy in business decision-making*
- I_{10}*: Cooperation of the company with domestic companies*
- I_{11}*: Cooperation of the company with foreign companies*
- I_{12}*: Cooperation of the company with public institutions*
- I_{13}*: Cooperation of the company with multinational enterprises*

The selected indicators will be subjected to descriptive statistical analysis. Principal Component Analysis (PCA) and Exploratory Factor Analysis (EFA) will then be used to reduce the indicators and extract the factors. Subsequently, based on empirical research and scientific knowledge from selected scientific papers, a model will be created through statistical Confirmatory Factor Analysis (CFA). The Chi-square test and additional fit measures in the form of Comparative Fit Index (CFI), Tucker-Lewis Index (TLI) / Non-normed Fit Index (NNFI), Goodness of Fit Index (GFI), Root Mean Square Error of Approximation (RMSEA) and Standardised Root Mean Square Residual (SRMR), will be used to evaluate the validity of the model. SPSS 26, AMOS 22, and JASP 0.14 software will be used to analyse the data. After verifying the created model's functionality through the mentioned fit measures, the results will be verbally evaluated, and the validity of the established hypotheses will be verified.

4. RESEARCH AND DISCUSSION

Thirteen manifest variables were chosen to verify the research hypotheses. At the outset, most of the representatives of the companies that took part in the questionnaire stated that they were working with other companies. Only eight companies out of the total number of 82 companies indicated that they did not cooperate with other companies, representing less than 9.8%, this means that more than 90% of companies collaborate with other entities. This result only confirms the findings of the research mentioned in the introductory part of the article. In 61% of cooperation cases with another company, this cooperation took place through informal communication, while in 39% of communication between companies took place on a formal level. The results of selected descriptive statistics can be seen in Table 1, which contains the number of observed values of individual variables, their mean value, standard deviations, and variances values.

Table 1 Descriptive Statistics

	N	Mean	Std. Deviation	Variance
I_1	82	1,27	0,786	0,618
I_2	82	3,66	0,593	0,351
I_3	82	0,98	1,721	2,962
I_4	82	3,38	1,348	1,818
I_5	82	4,96	1,281	1,641
I_6	82	4,77	1,408	1,983
I_7	82	4,93	1,235	1,525
I_8	82	4,82	1,306	1,707
I_9	82	3,88	1,767	3,121
I_{10}	82	0,72	0,452	0,204
I_{11}	82	0,51	0,503	0,253
I_{12}	82	0,5	0,503	0,253
I_{13}	82	0,2	0,399	0,159

Authors' work

Principal Component Analysis (PCA) and Exploratory Factor Analysis (EFA) were used to reduce the number of variables. The Exploratory Factor Analysis (EFA) evaluated reduced variables. The Exploratory Factor Analysis results were evaluated by the Kaiser-Meyer-Olkin (KMO) test and Bartlett's test. Afterwards, a model was created by Confirmatory Factor Analysis (FCA), the

functionality of which was subsequently verified. Due to PCA, EFA and CFA results, some variables were excluded from further analysis due to their statistical insignificance to the resulting model. These variables are I1: previous work experience of the business owner (executive manager) in the field of business, I2: Highest achieved education of the business owner (executive manager) and I3: Number of employees in the enterprise. The information that the employee's education does not affect its willingness to cooperate is in line with the other research findings [21]. Within the Principal Component Analysis, two factors were identified from the data. Due to empirical research related to the variables, the first factor was named the Liberalism factor, and the second factor was named the Strategy factor. The variables that are used in the models showing the factors influencing the willingness of a company in the creative industries to cooperate with their surroundings can be seen in Table 2.

Table 2: Cooperation Indicators

Factor	Manifest Variables
Factor 1 - Liberalism	I_8: Prevalence of teamwork in the company I_9: Democracy in business decision-making I_{11}: Cooperation of the company with foreign companies I_{12}: Cooperation of the company with public institutions I_{13}: Cooperation of the company with multinational enterprises
Factor 2 - Strategy	I_4: Gender composition of the working team I_5: Existence of goals that the company wants to achieve I_6: Existence of strategies and plans in the company I_{10}: Cooperation of the company with domestic companies

Authors' work

Based on theoretical knowledge and the results of Principal Component Analysis (PCA) and Exploratory Factor Analysis (EFA), a model was created through Confirmatory Factor Analysis (CFA). The model is shown in Figure 1. This model was then subjected to a closer analysis in terms of Additional Fit Measures.

In the case of the primary model fit indicator called Chi-square test, the value of $\chi 2$ of the test is 28.117, the number of degrees of freedom df = 26 and the value of p is 0.353, which is greater than the required value of p> 0.05. These results suggest that the model fits the measured data.

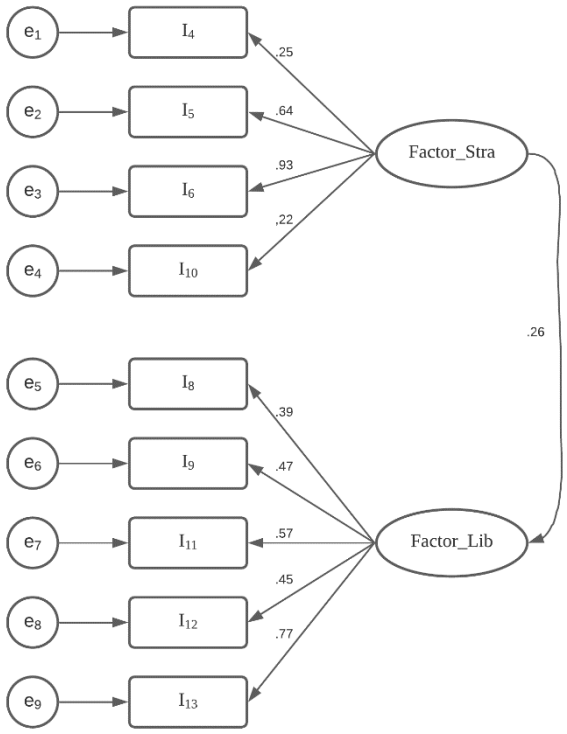

Figure 1: Cooperation Model (Authors' work)

The graphical interpretation of the model expresses the factor load's standardised values between the latent variables and the manifest variables, and the correlation coefficient between the two latent variables. In addition to the Chi-square test, the functionality of the model was also verified by additional Fit Indices, specifically through the Comparative Fit Index (CFI), Tucker Lewis Index (TLI) / Non-normed Fit Index (NNFI), Goodness of Fit Index (GFI), Root Mean Square Error of Approximation (RMSEA) and Standardised Root Mean Square Residual (SRMR). All the listed tests have exceeded the recommended minimum values; therefore, the proposed model can be accepted as a model that appropriately represents the analysed data. The results of individual Fit Indices can be seen in Table 3, together with the recommended values according to Hatcher [23].

Table 3: Characteristics of the model

Fit Indices	CFI	TLI/ NNFI	GFI	RMSEA	SRMR
Measured value	0.977	0.967	0.992	0.032	0.065
Required value	>0.90	> 0.90	>0.90	< 0.06	< 0.08

Authors' work

The model consists of two latent factors, which are the Strategy Factor and the Liberalism Factor. Each of these factors is made up of several manifest variables. The strategy factor consists of the output manifest I_4, I_5, I_6 and I_{10}. The liberalism factor is formed by the output manifest variables I_8, I_9, I_{11}, I_{12} and I_{13}. At the end of this part of the article, it is necessary to verify the established hypotheses. The results of the two hypotheses established in this article can be seen in Table 4.

Table 4: The results of hypotheses testing

	Hypothesized Relationship	**Estimate**	**Std. Error**	**p-value**
H1	F_STR → I_{11}	0.571	0.163	<0.001
	F_STR → I_{12}	0.445	0.150	0.003
	F_STR → I_{13}	0.605	0.200	0.002
H2	F_LIB→ I_{10}	0.291	0.215	0.176

Authors' work

H1: The higher the level of the company's strategic management is, the more positive effect it has on its willingness to collaborate.

The strategy factor has a positive effect on the variables I_{11}: Cooperation of the company with foreign companies, I_{12}: Cooperation of the company with public institutions and I_{13}: Cooperation of the company with multinational enterprises. The P-value is below the required value <0.05 in all three cases. Given the results, it is possible to reject the alternative hypothesis and accept the null hypothesis in its original wording. It turns out that the company's level of strategic management has a positive impact on the company's willingness to collaborate with its surroundings. According to the results from the created model, it has a positive effect on the company's willingness to collaborate with foreign companies, public institutions, and multinational companies.

H2: The more liberal management in the company is, the more positive effect it has on its willingness to collaborate.

In the case of the influence of the Liberalism Factor in the company on the variable I_{10}: Cooperation of the company with domestic companies, it is not possible to speak of a statistically significant effect, as the p-value significantly exceeds the recommended values <0.05. Therefore, in the second hypothesis, it is necessary to reject the null hypothesis and accept the alternative hypothesis.

5. CONCLUSIONS

The creative industries are a sector that differs in many ways from the more traditional sectors of the economy, for example, by specific internal company processes, a strong connection to critical personnel, or frequent rejection of purely economic logic. A company's willingness to collaborate with other companies or institutions is one of the main prerequisites for sharing knowledge and skills in this sector, which positively affects companies' innovative performance. Therefore, we consider it necessary to pay more attention to research at the company's microeconomic level. Only through such an approach will it be possible to understand the internal processes of the companies operating in the creative industries better.

The paper aimed to verify the established hypotheses that assumed a positive influence of latent factors in the company on its willingness to collaborate with other companies. The first hypothesis assumes that with the increasing corporate management level, the company's willingness to collaborate with companies will also grow. This hypothesis proved to be accurate, as the results of the Confirmatory Factor Analysis (CFA) in the form of a model, which was subsequently verified, show that the latent strategy factor affects the company's willingness to collaborate with other companies, especially to collaborate with foreign companies, public institutions, and multinational companies. In the first hypothesis, the null hypothesis is accepted, and the alternative hypothesis is rejected. The second established hypothesis, which assumed that with increasing liberalism of management in the company, the company's willingness to collaborate with other companies would also grow, was not confirmed. Thus, it is not possible to say that with the increasing liberalism of management in the company, its willingness to collaborate with its surroundings also increases. Therefore, in the second hypothesis, the null hypothesis is rejected, and the alternative hypothesis is accepted.

This research and the results of this paper can be considered as the basis for further research. It is necessary to verify the functionality of the model on a new data sample in the future. As already mentioned in the paper, it must also be borne in mind that the creative industries cannot be considered a homogeneous sector [7]. Therefore, it is not possible to consider the results valid for one subsector of the creative industries as automatically valid in other subsectors. The paper results were based on data obtained from the publishing industry, which is only one of many creative industries' subsectors. Therefore, it is necessary to verify the validity of this paper's findings in other subsectors of the creative industries as well.

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Putting into action crisis-driven business solutions during COVID-19 pandemic

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ABSTRACT

This research was conducted within the National Research project "ReCOVery - LV" for exploring the impact of the COVID-19 pandemic on the Latvian companies, specifically analyzing in this paper the range of emotions experienced by the Latvian companies and the new solutions they elaborated and realized to overcome the crisis despite the shock and stress. The qualitative content analysis of the feedback from 334 companies revealed their anxiety combined with the emotions of fear, anger and sadness, including six more emotions which present these three basic emotions' different levels of intensity. Although, being in this negative emotional state, some of the companies managed to increase their revenues introducing new products and services, creating new sales channels and attracting new clients, digitalize processes and achieve cost savings.

Keywords: crisis-driven business, emotions, new opportunities, COVID-19 pandemic

1. INTRODUCTION

The health necessitated lockdown and social isolation caused by COVID-19 have stopped most human and business activities which put forward unprecedented task to mankind to create new communication, working, economic, political, ecological and other living behaviors. In this revolutionary time to overcome the COVID-19 disaster, different anti-epidemic measures have been undertaken by many governments, businesses, health, educational and religious organizations involving in this battle against the pandemic also different societal groups and individuals. However, the attempts of governments to keep citizens at home and implement lockdown measures to slow down the spread of the virus, have turned out into another global pandemic – the pandemic of loneliness. This resulted in severe psychological stress of individuals around the world due to worries over the health, financial security, temporary unemployment, significant changes in our daily lives which all of us have to adapt to, studying, working and creating relationship with other people facing the lack of physical contact [1; 2; 3; 4].

In this uncertain and stressful reality doubled load is put on company owners and managers, as first of all, they are humans who also suffer physically and emotionally from this dramatic situation, and secondly, they bear full responsibility for their businesses which make the pillars of the economy of any country. Therefore, it is crucial to explore not only the new crisis-driven solutions which were put into action during the COVID-19 pandemic, but also the emotions felt by company heads for understanding of what kind of emotions could make driving forces for the innovative growth of companies in crisis. The key role of emotions in economics needs more attention to be devoted to [5], as traditional economic models built for rational and unemotional agents do not work effectively. Instead, new economic models of quasi-rational emotional humans should be built though it is a more challenging goal [6].
Accordingly, the research questions explored in this paper are:
1) What is the emotional state in the companies during the COVID-19 pandemic?
2) What business solutions are implemented to overcome the challenges caused by the COVID-19 pandemic?

2. EMOTIONS AND CRISIS-DRIVEN SOLUTIONS IN THE COVID-19 PANDEMIC

The COVID-19 pandemic has given a powerful boost to the digitalization of companies accelerating the innovating processes by replacing or complementing traditional businesses with new solutions. Companies have created many new offerings believing that to remain economically viable, they need to digitize their core business models [7]. It is argued that five years of e-commerce growth has condensed into three months due to the lockdowns and a shift in consumers' priorities, drastically reshaping both the consumer path to purchase and the actual points of purchase; that created new business patterns that most likely won't revert back to the pre-COVID-19 normal anymore [8]. Therefore, with a proactive view into the future, the fastest understanding of the post-pandemic generation is becoming one of the key factors of companies' competitive growth in recruiting, productivity, innovation and relations with customers [9]. It is believed that there will be a post-pandemic generation after the Z-generation (born in 1995-2012). If Z-generation is already

digital native and able to multitask while online, [10], there are not any characteristics known of the post-COVID generation. However, to meet the forthcoming demands with the most active involvement of the new generation-to-come, different digital business transformation models with the involvement of artificial intelligence are under active elaboration to: collect big data of business transactions; deploy big data analytics; ensure a better customer experience; design safe and contactless customer journey while acquiring the product or the service; and provide positive emotions via customers' enjoying the digital experience owing to powerful tools which will also capture and analyze customer behavior without human touch [11]. The understanding of emotions by artificial intelligence has a dual nature, as on the one hand, it excludes direct human-to-human interaction but, on the other hand, it is tended to preserve the focus on most human-specific characteristics – feelings and emotions in digital transformation of business models.

The great role of feelings and emotions (affect) in the entrepreneurial processes has been emphasized by scholars of the field, as feelings and emotions influence many aspects of cognition and behavior which might occur when entrepreneurs operate in unpredictable and uncertain situations performing many tasks and launching new ventures [12]. Being interconnected with each other, cognition and emotions impact the processes through which information is perceived, processed, stored and retrieved when needed for problem solving, innovation and decision making in highly dynamic entrepreneurial environments [13; 14].

It was proved that in stable and predictable situations emotions and feelings do not bring to such actions and decisions which they might cause in unknown or unstable situations [15]. And how can we characterize COVID-19 pandemic reality? With the emotions of worry, fear, stress, boredom, anger/annoyance [16; 17] causing anxiety, stress and depression [4; 18] threatening mental and psychological health of people worldwide [19]. People of different levels of emotional intelligence respond to the pandemic with different intensity of anger, disgust, sadness, fear, anxiety but not less frequently [20].

Despite the psychological support provided to vulnerable groups of the society, also in Latvia the level of depression, anxiety, insomnia and suicides have grown with the second wave of coronavirus [21], as people have become more aware of what COVID-19 is either having lost some of their relatives and friends or getting infected themselves and struggling against it. As the number of cases has grown more than 35 times in Latvia (calculated by the authors based on the Worldometer statistics) for the last four months (from October, 2020 to the end of January, 2020) [22], strict rules of anti-epidemic measures including curfew for weekends have been imposed due to the current state of emergency. All this unstable and uncertain situation worsens the emotional and psychological state in the society and creates additional challenges to businesspeople who not only worry about their own and their families' health and safety but also need to save their companies and find new working solutions to survive in the crisis. But is it possible to be creative and find new business solutions in such a negative emotional and psychological environment? The answer is 'yes', as also negative emotions and feelings can enhance creativity owing to individuals' efforts to attain creative outcomes [23]. Though, as concluded by Robert Baron, positive emotions and feelings enhance vs. negative emotions and feelings which reduce entrepreneurs':

- creativity needed for new opportunity recognition;
- capacity for acquiring essential financial and human resources;
- capacity for responding effectively to the highly dynamic environments they face;
- tendencies to expand both their skills and social network;
- adoption of relatively efficient strategies for making decisions;
- the capacity to tolerate high levels of stress, and this, in turn, may have beneficial effects on the health and wellbeing of entrepreneurs [24: 333-335].

In order to undertake any specific actions, individuals need to be motivated for that. Motivation has three internal sources - cognition, emotions and needs [25] which, "working together as a team", energize individuals to realize also entrepreneurial processes. Motivation has one more source - external events [25] both positive and negative, which in different ways activate the internal sources of motivation directing individuals toward definite behaviors and actions. The integrative driving force of cognition, emotions, needs and external events can enhance motivation but does not guarantee successful business performance, as there is one more crucial factor in-between – the entrepreneurial skills which play the role of a regulator [26]. Depending on the extent to which entrepreneurial skills are developed, businesspeople exhibit motivation to implement them in practice and succeed or avoid it [27].

Therefore, emotions have great impact on different aspects of the elaboration of business solutions and on the process of putting them into action. Also, the emotions experienced in COVID-19 are giving a boost to unprecedented development of innovation and new businesses similarly to the events in the mankind's history which showed that pandemics have crucial impact on the humanity development in biological, physical and economic dimensions.

Digital transformation is considered to be the most powerful strategic tool for turning the challenges caused by the COVID-19 crisis into new opportunities for businesses and all the aspects of the societal life [28, 11; 29; 30]. To survive and grow in the current conditions, for

companies it is essential to build new capabilities in weeks rather than in months or years [28; 30].

Having conducted empirical investigation, Bhattacharyya and Thakre concluded that company managers have simultaneously adopted dual approach responding to the crisis for surviving and growing both in short- and long-term perspectives correspondingly using reconfiguration of the existing resources and mobilization of the efforts for redesigning business models [31]. One of the paths to develop business models in this uncertain and unstable environment could be the stimulation and mobilization of the workforce to innovate and find new solutions to achieve companies' strategic objectives - revenue and profit growth, and cost reduction [32]. It is believed that to benefit even beyond the crisis period, companies have to invest in building new capabilities including digital infrastructure [30].

In this dynamic and often chaotic environment companies adapt to meet the changes in consumers' expectations, working remotely, adjusting supply networks, managing operations with the resources available, innovating across delivery channels, and redesigning product-portfolio based on the emerging new customer requirements to retain positive performance [31; 30].

3. NECESSITY IS THE MOTHER OF INVENTION: THE LATVIAN CASE

The survey questionnaire was structured of four parts to collect data on: a) the demographic characteristics of the companies which participated in the survey to get insight into the status of the respondents in their companies, the company age, location, sector of industry and number of employees; b) general impact of the COVID-19 pandemic on their businesses in regard with the opportunity of remote work, the number of employees fired, the extent to which COVID-19 has negatively impacted the companies and their turnover; c) specific external factors which have impacted and internal processes which have been affected by the pandemic, including also the opportunities identified and used for overcoming them; d) solutions undertaken by them to overcome the crisis. The questionnaire, which was designed to collect both quantitative and qualitative data, was elaborated in July and piloted in August, 2020. In the end of August, it was sent to Ltd Firmas.lv clients (n=2377) and members of the Latvian Chamber of Commerce and Industry and other associations. The response rate was not very high as only 334 companies shared their experience on the topic; more of it, only about half of them shared their views on open-ended questions. This can be explained with the overall shock experienced by companies in the face of the crisis and common reluctance of respondents to provide textual answers while sharing their experience.

The emotional state of the companies during the COVID-19 pandemic

The qualitative content analysis of the respondents' texts on how pandemic influenced their companies, along with the rational aspects, revealed also definite emotional components which were determined using as pre-constructed codes the emotions systemized by Robert Plutchik in the Wheel of Emotions [33]. It consists of eight (four pairs) of basic emotions: joy opposite to sadness; anger opposite to fear; trust opposite to disgust and surprise opposite to anticipation. Along with each basic emotion, there are two more emotions accordingly of lighter and stronger intensity of it.

Depending on the content of the responses, either one emotion or a combination of emotions was assigned to the text fragments in the process of coding. To illustrate in what way the qualitative content analysis was realised, a few fragments are given below in Table 1.

Table 1

A fragment of the qualitative content analysis

Text fragments	Emotions highlighted
My clients have lost all their businesses, and I have lost my clients!!!	Fear; annoyance; sadness
It has messed up absolutely everything. We have 80% reduction in turnover - all is bad!	Rage, grief, terror
I think the crisis is still ahead - there will be a drop in income, because the unemployment benefits provided in the crisis will soon end, and many other issues are waiting for us.	Fear, sadness, annoyance, remorse
We will be able to speak of the real impact in a year.	Pensiveness
In addition, because of having to buy sanitizers and pay for other precaution means, there is increase in costs.	Annoyance
I hate to comment on this "beastliness" called COVID!	Rage
No negative impact has been observed. On the contrary, some weak opportunities have emerged so unexpectedly.	Surprise, interest
It has not affected anything.	No emotions

The qualitative content analysis resulted in three groups of emotions shown in Table 2. The basic emotions of these groups are anger, fear and sadness. As for the less intensive (annoyance, apprehension and pensiveness) and more intensive (rage, terror and grief) emotions within each of

the three groups, they are shown correspondingly in lighter or darker shades.

Table 2
The three groups of emotions expressed by the company owners, business managers and heads of structural units related to the impact of the COVID-19 pandemic on their companies

Basic emotion	Emotion	Absolute frequency	Frequency (%)	Frequency (%)
Anger	Annoyance	90	31.40	34.10
	Anger	3	1.00	
	Rage	5	1.70	
Fear	Apprehension	57	19.90	30.70
	Fear	18	6.30	
	Terror	13	4.50	
Sadness	Pensiveness	36	12.50	30.30
	Sadness	37	12.90	
	Grief	14	4.90	
No emotions	No emotions	14	4.90	4.90
Total		287	100	100

The total frequencies of all the three basic emotion groups are very close to each other – anger (34.10%), fear (30.7%) and sadness (30.30%) which speaks of these emotions being similarly typical of the surveyed companies. Still, most frequently among all the nine emotions (see Table 2) was mentioned annoyance (n=90; 31.40%) - the lighter intensity of anger. The frequency of apprehension (n=57; 19.90%), the lighter intensity of fear makes almost one fifth of the total number of frequencies. In the third group the basic emotion – sadness (n=37; 12.90%) and its lighter intensity - pensiveness (n=36; 12.50%) were highlighted similarly frequently. The stronger intensities of these negative emotions – rage (n=5; 1.70%), terror (n=13; 4.50%) and grief (n=14; 4.90%) were recognized significantly less frequently in the course of the qualitative content analysis.

Some other emotions felt and shared by the respondents did not even form categories because of their low frequency – surprise (n=1), interest (n=1), remorse (n=1). Emotionally neutral responses - no emotions (n=14; 4.90%) were given only by a small group of respondents. This is an evidence of serious emotional tension among the Latvian company owners (53.62%), business managers (24.64%), heads of structural units (13.77%) and others (5.80%).

The solutions found by the companies to overcome the crisis caused by the COVID-19 pandemic

Despite the atmosphere of the entire shock, distress and anxiety, some of the companies managed to find new innovative solutions to overcome the crisis and even improve their companies' work efficiency. The qualitative content analysis of the responses to the question on the opportunities identified and used by the companies with a positive impact on their businesses during COVID-19 revealed three groups of categories related to: a) increase in revenues caused by new product and service introduction, creation of new sales channels and attraction of new clients; b) digitalization of processes owing to new IT solutions, new forms of work organization, introduction of new internal and external communication forms, new forms of document turnover; c) cost savings because of introduction of IT solutions and attracting new specialists at a reasonable price conditioned by increasing their competitive advantages. As a result, increase in work efficiency was observed (see Fig. 1).

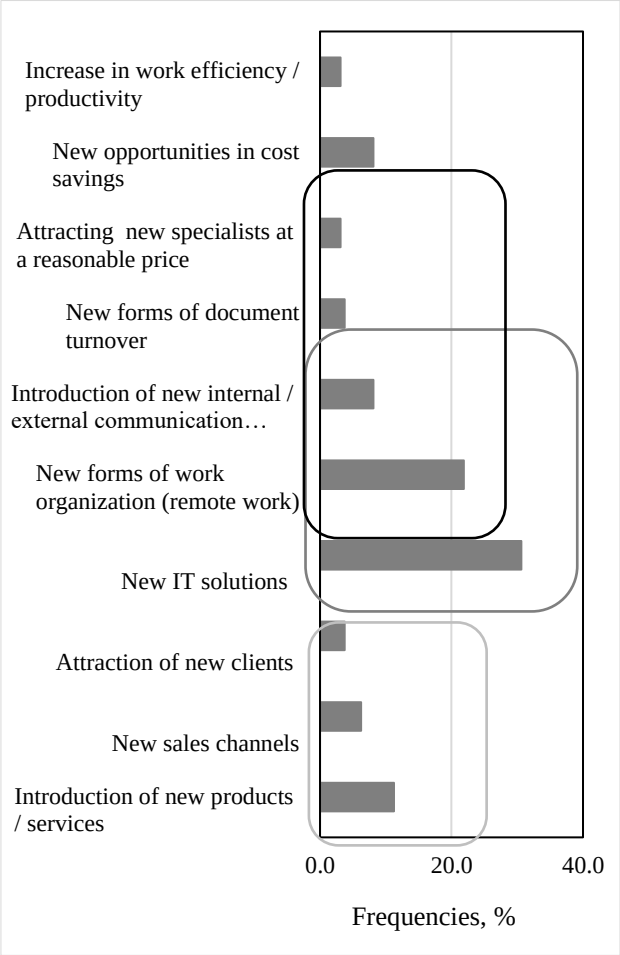

Figure 1. The distribution of frequencies of new opportunities and business solutions identified and put into action by the companies during COVID-19

There are also other types of new opportunities identified and used by the companies while searching innovative solutions through introduction of new technologies, new opportunities for brand development, e-learning of clients, improvement of the infrastructure, the finding of new

collaboration forms in the company, self-production of raw materials and optimization of the production process.

4. CONCLUSIONS

Initially this research project was not designed to explore the emotions experienced by company owners, business managers and heads of structural units of the Latvian companies caused by the COVID-19 pandemic. However, along with respondents' rational answers, also their emotional reaction given to open-ended questions made us pay a special attention to this side of the reality. Therefore, we decided to analyze not only the crisis-driven solutions which were put into action by the companies but also the emotional background against which all these activities were developed and realized.

When people face real threats, uncertainty or the unknown, their normal responses to them are fear, worry and stress [1]. A similar set of negative emotions – anger, fear and sadness, were almost equally experienced by the companies surveyed. In our research these basic emotions were revealed to be combined with the emotions which make accordingly their lighter (annoyance, apprehension and pensiveness) or stronger (rage, terror and grief) intensities. No emotions were demonstrated only by very few respondents - less than 5%; this speaks of the overall negative emotional state of the majority of the businesspeople who took part in this research. However, the research also revealed that one third of the total frequencies made annoyance - the lighter intensity of anger, and one fifth – apprehension – the lighter intensity of fear, while the total frequencies of rage, terror and grief - the stronger intensities of accordingly anger, fear and sadness, made about one tenth of the total frequencies (see Table 2).

Even if we try to see some optimistic signs in this proportion of the lighter and stronger intensities of these negative emotions, obviously, this is not the case considered by Baron when owing to positive emotions, there usually can be observed enhancement in entrepreneurs': creativity needed for new opportunity recognition; capacity for acquiring essential financial and human resources; and efficient decision making in highly dynamic environments they face [24]. On the contrary, the new crisis-driven business solutions were elaborated and put into action owing to the companies' big intellectual and organizational efforts to attain creative outcomes despite the anger, fear and sadness. So, these findings prove the conclusions made by George and Zhou [23].

The nine new business solutions elaborated and put into action have logical interconnection which could be understood via consideration of the three groups of new opportunities: increase in revenues; digitalization of processes owing to new IT solutions; and cost savings (see Figure 1). As a result, increase in work efficiency and productivity has been achieved. These solutions were mainly realized by experienced small companies with the employees' number not exceeding 50 and with the company age over 18. Regardless of the surveyed companies' age and number of employees in them, speaking of the key success factors, all of them emphasized the optimization, digitalization and investment of new technologies.

Thus, the new business solutions elaborated and put into action by the surveyed Latvian companies go in line with responses towards the COVID-19 pandemic adopted by other companies worldwide [28; 29; 30; 31; 32]. So, one more time necessity was proved to be the mother of invention.

5. ACKNOWLEDGEMENT

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Organizing Studies Entrepreneurially to Adjust Inner and Outer Worlds during COVID-19

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ABSTRACT

This research aims to analyze the effect of a five-stage strategy elaborated within the Erasmus+ project FOrSE (Framework for Organizing Studies Entrepreneurially) realized by three EU universities. The strategy was integrated into a study course of Pedagogy to enhance entrepreneurial mindsets and behaviors of first-year international Master students who did not have any opportunity for face-to-face studies because of the quarantine. The key focus of the strategy is to promote students' ability to analyze the problems facing them during the COVID-19 pandemic and identify and/or realize new opportunities in the face of the worries and uncertainty. The qualitative content analysis of the Master students' reflections and the analysis of the challenges faced, and new opportunities identified and/or realized showed that they tried to adjust their inner world to the events which take place in the outer world for gaining self-confidence and achieving harmony.

Keywords: organizing studies entrepreneurially, inspiration, opportunity identification, opportunity realization, self-negotiated action, COVID-19

1. INTRODUCTION

The uncertainty about today and tomorrow, drastic changes in lifestyle and dramatically growing consequences of COVID-19 worldwide have brought into question almost all the aspects of life organization and values. One of the crucial issues is about education and the way it should be organized to promote the 21st century competences to help learners enhance their self-confidence in the rapidly transforming world without getting lost in the life labyrinths, feel strong and whole, be in dialogue with their own selves and the outer world. Its importance cannot be overestimated in the reality of considerable degree of fear, worry and concern induced by the coronavirus pandemic threatening not only physical but also mental health of people [1].

One of the ways of accomplishing this complex task could be the organization of studies entrepreneurially whose ultimate goal is seen in helping learners to become self-negotiated actors who are capable to "direct their conscious thinking and action towards an adjustment of their inner and outer worlds in order to succeed in life" [2:58]. Emphasizing the topicality of such learning outcomes for all students regardless of their specializations, the authors suggest that the accent should be shifted from 'studying for entrepreneurship' to 'studying entrepreneurially' by embedding opportunity-centered learning strategies into general educational practice even for learning study disciplines traditionally not related to business and entrepreneurship. This will enhance the quality of higher education enabling to overcome the five learning gaps of university students between: 1) recall and understanding, 2) understanding and ability, 3) ability and wanting to; 4) wanting to and actually doing, 5) actually doing and ongoing change, highlighted by [3]. Organizing studies entrepreneurially tends to promote students' development-oriented entrepreneurial mindset across broad pedagogical practice and contexts and giving a boost to such essential entrepreneurial skills as: courage, responsibility, initiative, tolerance for ambiguity, interactivity, and ability to collaborate and creativity [4]. Such an organization of teaching and learning process enhances the practical value of education as it is linked to real life situations, problem solving, and new value creation inspired by the environment we live in. More of it, it triggers several active learning channels which accelerate not only the development of students' multiple skills, cognitive power and creative behavior but also increases their motivation to become more involved in such a learning process, causing them new positive emotions, opening new horizons for development and disclosing new bigger needs to be analyzed and met in further studies regardless of the study disciplines [5]. Hence, working entrepreneurially should not be seen only as dealing with business, but more widely for making learners initiative, cooperative, responsible and creative decision makers. Therefore, it is argued to be beneficial to conduct a research which would be the most appropriate way to bring out the relationship in business and entrepreneurial behavior, especially into non-business education at different levels [6; 7]. However, the new reality of the COVID-19 pandemic throws additional challenges to this research area as it is more practice-based but the present style of living in social isolation and lack of opportunities for realizing ideas in physical vs. virtual cooperation, make the organization of studies entrepreneurially a more complicated task. That is why, this research focuses exactly on the exploration of the opportunity of organizing online university studies in such a way which could light sparks of hope, optimism and self-confidence driving out hopelessness, pessimism, and frustration caused by the overall uncertainty and anxiety.

The research questions explored are:

1) What challenges do students highlight in COVID-19?

2) What opportunities for personal growth and new businesses do students identify?

The research findings are based on the:

- analysis of Riga Technical university first-year international Master students' projects elaborated and realized by them being in the quarantine and social isolation because of the COVID-19 pandemic;
- qualitative content analysis of the students' reflections on what they acquired within the project and how their attitude towards life difficulties, their thinking, acting and perception of life have changed while realizing the opportunities identified.

2. THE THEORETICAL FRAMEWORK FOR ORGANIZING STUDIES ENTREPRENEURIALLY

Despite a great deal of contradictions, theories and counter-theories related to the matter, structure and functions of entrepreneurship, there is one undeniable point of view shared by the majority of scholars of the field – that one central activity is opportunity identification and development [8; 9; 10]. Therefore, to enhance entrepreneurship education, different learning strategies and models have been elaborated for: opportunity exploration and realization [11]; opportunity recognition using cognitive, behavioral and action learning tools [12], opportunity identification and exploitation using creativity-based experiential learning model [13], etc. A crucial role in opportunity discovery is played by prior experience and search [14; 15], which are tightly linked to the process of the development of knowledge stored in the heads of entrepreneurs as cognitive structures created via interaction with the world around them [16; 14; 17]. These cognitive structures are afterwards processed, stretched, expanded or combined to generate new ideas [18; 19]. So, the more students create cognitive structures and generate new opportunities in their study process, the more they will think and behave like entrepreneurs and they will be able to apply such skills across a broad range of situations [20].

It is believed, that while passing through such a learning process, students develop also topical skills that can help them become more capable of self-negotiated action [2] and lead a successful and fulfilling life. Therefore, if we shift the emphasis from entrepreneurship education to organizing studies entrepreneurially even within traditionally non-business-related study disciplines like physics, biology, arts, chemistry, music, literature, etc., opportunity identification, generation, development and realization ought to become an inseparable part of broad pedagogical practice which implies:

- recognition of new potential, ideas and opportunities derived from the study content and context [21; 22; 23];
- having clear understanding of how knowledge and skills acquired within and across study disciplines can be applied to solve real-life problems in multiple ways for creating new values for oneself and the society [24];
- enhancement of self-confidence based on the regular practicing of getting oriented in the changing study environment [6];
- facing challenges and overcoming them becoming more inspired learners [25].

Inspiration is needed for entailing motivation, energizing and directing behavior towards a desired target using triggers, such as persons, ideas or events [26]. Therefore, inspiration plays a key role as a starting stage in this learning and research project meant for the students to generate cognitive ideas based on their experiences, evaluate and test them in practice [27].

As for the concepts of inner and outer worlds, in the scientific literature they are defined and understood in various ways. In some sources the inner and outer worlds are given as concepts that fix the difference between everything that refers to the phenomena of the human mental sphere (the inner world), and those that do not belong to it (the outer world) [28]. It is believed that the inner world is also reality, but of a different order; more of it, the knowledge of the outer world remains imperfect without knowing the inner world of a person. The inner world contains all the diversity feelings, sensations, images, meanings of the human mental spheres that are capable of directing human life and influencing the outer world [29]. Hence, also cognitive processes which are needed for learning, gaining experience and skills, as well as metacognitive processes which are essential for controlling, analyzing, organizing, constructing and improving the thinking process are constituent parts of the inner world as they belong to human mental sphere. Therefore, in this research psycho-emotional, self-organizational and self-developmental aspects of the challenges and opportunities identified by the students are related to their inner world. Meanwhile, economic, infrastructural and socio-organizational challenges and opportunities linked to the life organization in the society are related to the students' outer world (see chapter 3).

3. THE FIVE-STAGE STRATEGY FOR ORGANIZING STUDIES ENTREPRENEURIALLY

The research strategy combined learning activities according to the model of organizing studies entrepreneurially with data collection conducted in the spring semester of 2020. The Riga Technical University Master students who participated in this study and research process were specialized in: Computer Systems, Business Informatics, Smart Electronic Systems, Telecommunication, Construction Business and Real Estate Management, Electronics, Entrepreneurship and Management, Aviation transport and Mechanical Engineering. The students came from India, Sri-Lanka, Pakistan, China, Azerbaijan, Lebanon, Egypt, Mexico, and Nigeria. They had just arrived in a new country which was thousands of kilometers away from their native lands and become locked in the dormitory rooms without having the opportunity for getting socialized or moving freely for getting acquainted with the new culture and their new place of living. They were to start Pedagogy as an optional study course just before the quarantine. Therefore, the study course was realized only through online and video lectures, online consultations, and individual projects. Seeing how lost and depressed the students were in the beginning, it was decided to inspire them by the study material "Every cloud has a silver lining" elaborated within the theme "The 21st century competencies" (stage 1). It was perceived very positively by the students; therefore, they were given a task to elaborate and realize their own individual projects passing through stages 2, 3, 4, and 5 of the model of organizing studies entrepreneurially described below:

1. **"Inspiration"**. Analysis of some materials aimed to inspire students with life stories of famous people from different fields – entrepreneurship, music, sport and writing, who faced serious challenges but overcame them becoming very wealthy and successful owing to: positive thinking; creativity; ability to find ways out of even perplex

situations; talent for seeing perspectives of growth; purposeful and systematic work, etc.

2. **"Opportunity identification".** Students' analysis of the challenges faced by them and recognition of opportunities which could be derived from these challenges. Choosing the most inspiring opportunity from the list and setting up a goal for doing something valuable for themselves or others.
3. **"Opportunity realization".** Elaboration of individual projects by the students and their implementation for achieving the goals with day-to-day registration of the activities and the progress in a diary.
4. **"Performance analysis".** Writing a report by the students analysing their own outcomes providing photos/videos if possible.
5. **"Self-analysis".** Students' reflection on what they understood while realizing the project and what has changed in their: thinking, attitudes towards difficulties and challenges, perception of life, and behavior.

The students' reports made the body of the empirical data; after receiving all the reports, their parts were reorganized into an excel file and prepared for the qualitative content analysis to answer the research questions conducting open coding for inductive category development [31].

The challenges highlighted by the students during the quarantine

The number of challenges indicated by each student in his/her report varied from 3 to 8 (mean = 4.93; median = 5) but in some cases due to their complex nature, the qualitative content analysis (QCA) exposed more than one challenge combined in one.

Table 1
The challenges related to the students' inner world

	Domains	Category	Frequency	Sum of frequencies	
Inner world	Psycho-emotional	Apathy, boredom & laziness	19	58	108
		Fear	15		
		Depression & frustration	12		
		Feeling lonely & lost	7		
		Missing family & homesickness	5		
	Self-organizational	Poor self-disciplining	16	50	
		Disturbed daily routine	10		
		Disorganization of diet	10		
		Forming undesirable habits	7		
		Passive & unhealthy lifestyle	7		

The subsequent analysis of the 21 categories developed in the QCA revealed their relation to five domains of challenges; two of these domains – 'Psycho-emotional' (n=58) and 'Self-organizational' (n=50) are relevant to the students' inner world controlled by them (see Table 1), and the rest three domains – 'Socio-organizational' (n=79), 'Economic' (n=47) and 'Infrastructural' (n=23) – relevant to the outer world not controlled by them (see Table 2).

Together with the categories of Fear (n=15) and Depression & frustration (n=12) which were experienced almost by everybody living in the conditions of strict social isolation, there were also psycho-emotional challenges specific for this group of students who found themselves in a long-term quarantine just having arrived in Latvia. Accordingly, all this caused apathy, boredom and laziness (n=19), intensified with the feeling of loneliness and being lost (n=7) combined with missing family and homesickness (n=5) (see Table 1). Consequently, this state of mind and emotional decline brought to failures in self-organization. The students mentioned that they had challenges of self-discipline (n=19), as they could not always organize their daily routine (n=10) because of irregular sleeping and eating, playing computer games, watching films at nights, having long skype chats, etc. In addition, they had difficulties in organizing their diet (n=10) as many of them (male students) could not cook. This passive and unhealthy lifestyle (n=7) was gradually forming undesirable habits (n=7) weakening the inner world of the students.

Table 2
The challenges related to the students' outer world

	Domains	Category	Frequency	Sum of frequencies	
Outer world	Socio-organizational	Staying indoors	25	79	149
		Missing travel opportunities	15		
		Distance learning	11		
		Social distancing	11		
		Sedentary lifestyle	9		
		Anti-virus measures	8		
	Economic	Job related problems	24	47	
		Financial difficulties	18		
		Rise of grocery prices	5		
	Infrastructural	Public transport	15	23	
		Restricted shopping opportunities	8		

As it was the spring semester of 2020 and the full distance learning was at its initial state of development, the students complained of experiencing lack of human interaction with their groupmates and teachers which negatively impacted their learning outcomes. Along with the dislike of full distance learning (n=11), the students were unhappy also with other aspects of life organization in the society (n=79) in that period (see Table 2) which came into their reality from the outer world.

One of the most painful challenges among them was the missing travel opportunities (n=15); it turned out that these young people were active travelers who suffered of the sedentary lifestyle (n=9). The students faced serious economic challenges, as they planned to combine university studies with job which was too hard for the realization (n=24); that caused financial difficulties (n=18) worsened by the rise of food grocery prices (n=5). The restricted shopping opportunities (n=8) and the safety measures in using public transport (n=8) were infrastructure related challenges which greatly irritated the students.

Thus, the students highlighted more frequently challenges coming from the outer world (n=148) compared to the challenges conditioned by the state of their inner world (n=108) (see Tables 1 and 2).

The opportunities for personal growth and new businesses identified by the students during the quarantine

As the time given for the realization of the project was from 15 days to one month, the students had enough time to undertake some activities which brought to tangible results. Since the opportunities derived from the challenges faced, it was not surprising that they had some internal logical connection with the 'Psycho-emotional' (n=47), 'Self-organizational' (n=100), 'Socio-organizational' (n=29) and 'Economic' (n=21) domains (see Table 3).

However, no opportunities were identified related to 'Infrastructural' domain as the students could neither impact any aspects of public transport nor the weakening of shopping restrictions nor any infrastructure related aspect of the new place of living. At the same time a new 'Self-developmental' (n=110) domain was formed which turned out to be the most frequently mentioned one (see Table 3).

To overcome the psycho-emotional challenges, the students started practicing different hobbies (n=12), meditation (n=11), yoga (n=8), positive thinking exercises (n=4), inventing also different ways of entertainments (n=12) like virtual travelling, online picnicking with families and friends accompanying that with music, games, food and a lot of fun.

The opportunities identified and realized within the self-organizational domain had a very positive impact on the students as owing to them they managed to overcome the challenges mentioned within that domain – even the male students learnt to cook (photos and in some cases even the recipes of their cooking masterpieces were given in the project report) and became independent from cafes and canteens (n=27); owing to regular exercising (n=21), healthy lifestyle (n=9) and well-organized diet (n=14) some of the students managed to get rid of extra kilograms gained because of the unhealthy lifestyle (they provided their before-and-after photos); instead of useless chats and internet night serials, step by step they created new routine to go to bed and wake up in time and manage all the daily routine and university assignments according to the schedule without procrastination (n=8); some of them even managed to analyze their expenses critically and reorganize their personal finances in such a way that they got rid of their debts and managed their finances more effectively (n=5); some students created plans for their lives having analyzed themselves, their experiences, likes and dislikes (n=5).

The 'Self-developmental' opportunities identified and realized by the students make the third domain (n=110) of the group of opportunities related to the students' inner world (see Table 3). Majority of the students devoted more energy and time to the learning of different new things important for themselves (n=31); (n=16) showing the certificates got in the end of that period.

Table 3

Opportunities identified by the Master students

Direction	Domain	Categories	Frequency	Sum of frequencies	
Inner world	Psycho-emotional	Practicing hobbies	12	47	257
		Entertainment	12		
		Meditation & mental health	11		
		Yoga	8		
		Positive thinking	4		
	Self-organizational	Cooking	27	100	
		Exercising & sporting	21		
		Organization of diet	14		
		Healthy lifestyle	9		
		Self-disciplining	8		
		Organization of daily routine	8		
		Personal finance management	5		
		Life planning	5		
	Self-developmental	Learning	31	110	
		Enhancing creativity	27		
		Improving professional skills	16		
		Enhancing talents & abilities	14		
		Reading	12		
		Self-discovery	10		
Outer world	Socio-organizational	New value creation	11	29	50
		Spending time with family	9		
		Combating social distancing	9		
	Economic	New business ideas	21	21	

Some students got inspired to such an extent that they decided to realize their dreams (drawing, dancing, creating internet blogs, composing songs, growing flowers and lettuce on the windowsills, writing stories, reading science fiction) which before were ignored or couldn't be devoted time to because of different reasons. Thus, the students enhanced their talents and abilities (n=14) and made self-discoveries (n=10).

All these opportunities are not business-oriented but the students' inner-world-oriented. They will not bring directly or at once to the creation of some values for commercialization today, but they are key factors for making students' whole, physically and mentally balanced and happy in the face of the COVID-19 pandemic.

However, there were also opportunities identified and initiated to impact the outer world (n=50) such as: new value creation (n=11) - creating new types of learning courses on coding; elaborating new video-game concept; making sanitizers at home using homemade natural and harmless ingredients (ayurvedic leaves like tulasi, neem and others), etc. The students purposefully started to seek new more fruitful ways of spending time with families (n=9) and combating social distancing (n=9) with friends; some of them even managed to elaborate business plans through such skype meetings for starting businesses with families or friends or improve the already existing ones (n=21).

Thus, the challenges faced by the students made them identify and realize new opportunities for overcoming them. Nonetheless, the profiles of the distribution of frequencies of the six domains for the challenges and opportunities are different (see Figure 1).

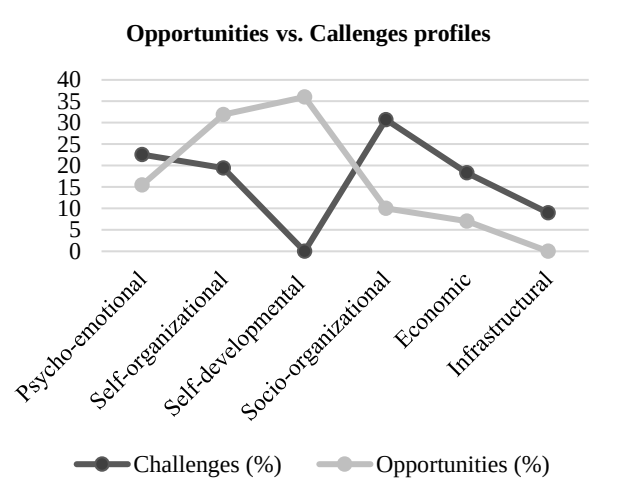

Figure 1. The distribution of frequencies of the domains of opportunities and challenges

This means that the numbers of opportunities identified related to each domain do not correlate with the number of challenges mentioned by the students. In this research such a discrepancy of the profiles does not play any special role, as a summative effect of challenges which brought to opportunity identification is obvious. As for the 'Self-developmental' domain of opportunities (this did not emerge among the domains of challenges), it could be considered as subpart of the 'Self-organizational' domain, as it became possible owing to the activities tended to organize the students' daily life. Still these two domains were not integrated in one with the intention to lay a special emphasis on the huge positive effect of the students' self-organization.

Summing up all the frequencies of the challenges and opportunities related to the inner and outer world, it was concluded that more challenges were mentioned coming from the outer world while more opportunities were identified and realized for the perfection of the inner world (see Figure 2).

This finding is very typical for humans as they usually see more problems coming from outside rather than they recognize them in their inner-world conducting critical self-analysis. And when they try to do some good things, first of all they think of their personal fulfillment and only then they direct their efforts to the perfection of the outer world.

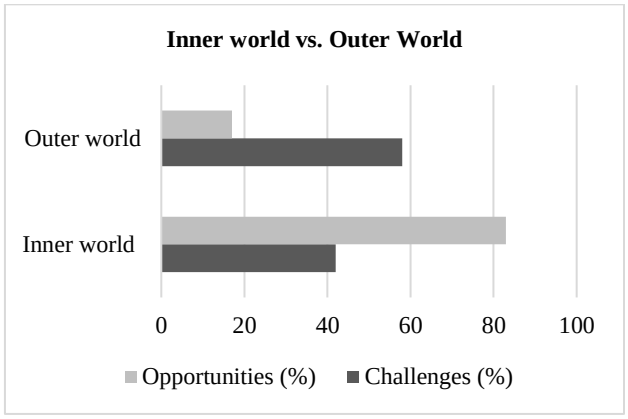

Figure 2. Analysis of the opportunities and challenges related to the students' inner world vs. outer world

4. CONCLUSIONS

This was an attempt to integrate and explore the effect of the five-stage strategy for organizing studies entrepreneurially into a university study course of Pedagogy relating that to the theme "The competencies topical in the 21st century". Without expecting each student to become an entrepreneur, it was rather hoped to offer students another strategy for active learning by doing, reflecting, identifying opportunities even in the crisis, solving problems for realising them into new values for themselves or the society - exactly in the way entrepreneurs think and act. By doing so, the students were supposed to adjust their inner world to the outer world for disclosing and strengthening their potential becoming more organised and enhancing their awareness of their own state of mind, talents, and dispositions. It is not excluded that for some of the students the discovery of their talents could turn into a base for businesses in the future, but this will be a problem for some other research.

The characteristic feature of the five-stage strategy for organizing studies entrepreneurially is in its first stage - 'Inspiration' which worked as trigger for self-developmental opportunities. The stories about people who overcame serious challenges and achieved the peak in their career energized the non-entrepreneurship students and gave them a boost enhancing their confidence that also they themselves could overcome the stress and crisis caused by COVID-19 pandemic and realize their potential and find opportunities for creating new values for themselves and others.

The findings show that the challenges highlighted by the students gave birth to opportunities identified and realized by the students deriving from these challenges. The challenges and opportunities which pertain both to the students' inner world are within psycho-emotional and self-organizational domains and their outer world – within socio-organizational and economic domains. As for the opportunities which can be related to the self-developmental domain, here they are considered as a separate group because of their high weight (n=110) compared to the other domains, though these opportunities make logical consequences and outcome of the students' self-organization.

Thus, the five-stage strategy of organizing studies entrepreneurially could be considered as an appropriate approach for promoting students' self-negotiating actions as it directs learners' conscious thinking and actions towards an adjustment and balancing of their inner and outer worlds in order to succeed in life. However, more research ought to be conducted with the

embedding of this strategy also in the regular practice of other university study courses for the generalization of this pedagogical approach.

5. ACKNOWLEDGEMENT

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Analysis of Cerebral Blood Flow Complexity when Listening Music with Emotional Content

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ABSTRACT

Despite differences in ethnicities, culture, or language, music is a universal stimulus that can evoke intense feelings in people. Studying its effects, however, is challenging because of its emotional nature. This study analyzes the effects of listening different sound stimuli, such as music with emotional content, on cerebral hemodynamics. Cerebral blood flow signals were recorded for 16 subjects while performing five different music tasks. The complexity of each signal was estimated using multiscale Sample entropy. Significant differences in mean complexity was found between two tasks, which suggests that intense cognitive activities having emotional content yield a decrease in the complexity of cerebral hemodynamics.

Keywords: Complexity, Multiscale Entropy, Cerebral Blood Flow, Music, Hemodynamics, Sample Entropy.

1. INTRODUCTION

Music is one of the most sublime stimuli that human beings can experience. Despite being just an acoustic wave that exerts little physical action on a subject, it triggers profound changes in emotions and physiological states. Indeed, several experiments have sought an explanation for this phenomenon at the neuronal level [1].

One study [14] analyzed (EEG) signals collected from 26 healthy subjects listening to different music using Support Vector Machine (SVN) classification to distinguish four emotions (joy, anger, sadness, and pleasure) according to two-dimensional circumplex emotion model comprising valence (positive-negative) and arousal (high-low) axes.

Another study [15] also utilized EEG to compare the levels of signal complexity with the musical complexity that subjects were listening to. They were required to do two tasks: (i) closely track the acoustics (perceptual task) and (ii) think about how the music made them feel (emotional task). Multiscale entropy (MSE) was used to determine complexity matching (effectiveness of the brain to reflect the content of the environment information) between EEG and music signals.

There is another study [16] that examines audible EEG signals recorded from subjects both in rest state and hearing music stimuli (tanpura drone) to analyze the correlation between both sounds using a microscope called Multifractal Detrended Cross-Correlation Analysis. Results showed there was a rise in correlation as the audio clip progressed and that music stimuli had the ability of activating many brain regions parallelly or in different moments.

There are several complementing investigations. In [3] the researchers asked subjects to listen to consonance and dissonance recordings while measuring their cerebral blood flow (CBF), using a positron emission tomography, to examine correlations between consonance levels and emotions, finding that music might engage neural mechanisms associated with pleasant or unpleasant emotional states. Research in [4] looked at the effects of musical experience on hemispheric lateralization using magnetoencephalography, whose results indicated that musical training changes the hemispheric roles for musical feature processing. Finally, the work in [10] sought changes in CBF velocity (CBFv), by using transcranial Doppler ultrasound, in response to emotional stimuli on heathier subjects.

Despite the above studies, there is a lack of research on the effects of music on cerebral hemodynamics. This is fundamentally due to limitations on the analysis of CBF signals, that usually is limited to inspect mean amplitude or spectral density, which have not provided clear results on differentiating emotional states. However, nowadays there are powerful tools that allow signal analysis from a complexity perspective that have found successful applications with biological data.

This study proposes that analyzing the hemodynamic response to different musical stimuli using a complexity estimator derived from MSE will properly differentiate between the emotional reactions they trigger.

2. BACKGROUND

Sample Entropy

Sample Entropy SampEn is a method for the estimation of the repeatability of predictability within a time series that has been used to characterize physiological signals from a number of imaging modalities.

SampEn definition considers $A_i^m(r)$ and $B_i^m(r)$ [5, 6]:

$$A_i^m(r) = \frac{1}{N-m-1}\sum_{j=1,j\neq i}^{N-m}\begin{bmatrix} number\ of\ times\ that \\ d[|x_{m+1}(j) - x_{m+1}(i)|] < r \end{bmatrix} \quad \text{Eq. (1)}$$

$$B_i^m(r) = \frac{1}{N-m-1}\sum_{j=1,j\neq i}^{N-m}\begin{bmatrix} number\ of\ times\ that \\ d[|x_m(j) - x_m(i)|] < r \end{bmatrix} \quad \text{Eq. (2)}$$

In which N specifies the number of observations, r is a tolerance value to filter noise, m is the pattern length, x_i and x_j are two blocks on time series of length N.

Then, the following expressions are presented:

$$A^m(r) = \frac{1}{N-m}\sum_{i=1}^{N-m} A_i^m\,(r) \quad \text{Eq. (3)}$$

$$B^m(r) = \frac{1}{N-m}\sum_{i=1}^{N-m} B_i^m\,(r) \quad \text{Eq. (4)}$$

Finally, SampEn is defined as:

$$SampEn(m,r) = \lim_{N\to\infty}\left\{-\log\left[\frac{A^m(r)}{B^m(r)}\right]\right\} \quad \text{Eq. (5)}$$

The two parameters, namely the length of the segments to be compared (m) and the tolerance for accepting matches (r), are critical to the performance of MSE. As there are no definitive guidelines on how to choose their values [7], all combinations of (m, r) parameters will be analyzed as explained below.

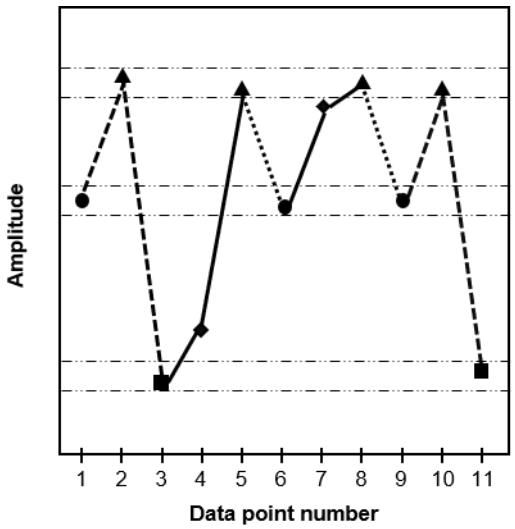

Fig. (1). Simple time series example with representations of the parameters used for SampEn calculation. Using pattern length m = 2, sequences of m and m + 1 (i.e. with length 2 and 3) are collected as templates. r value is represented as horizontal dotted lines around circular, triangular, and squared data points symbolizing points that match each other. Thus, the same sequences of length 2 are found between data points 1-2 and 9-10, 2-3 and 10-11, and 5-6 and 8-9. The same sequences of length 3 are found for data points 1-2-3 and 9-10-11.

The example signal of Fig. (1) will be used as a running example to explain the SampEn calculation.

First, sequences of length m and m + 1 are extracted from the signal. Two sequences are considered to be the same pattern if the values of each of their corresponding data points are within a distance ±r. In Fig. (1), for example, the sequence observed in data points 1-2 matches the sequence formed by data points 9-10. Analogously, sequences 2-3 and 5-6 can be matched to sequences 10-11 and 8-9 respectively. Similarity, the sequence of length 3 composed of data points 1-2-3 can be considered to be the same pattern as the sequence 9-10-11. The degree of repetition of these patterns can be used to assess the predictability or regularity of a signal.

SampEn is the negative natural logarithm of the conditional probability that two different sequences of length m with the same pattern remain similar at length m+1 [6]

Multiscale Entropy

MSE is a method to calculate entropy on complex signals, considering the multiple time scales inherent to biological systems [2].

Although MSE applies to many entropy measures, wherein this study SampEn is utilized.

MSE analysis comprise two steps. In the first step, several coarse-grained time series $\{y(\tau)\}$ are built from the original time series $\{x_1, \ldots, x_i, \ldots, x_n\}$ by averaging τ successive incremental number of points in non-overlapped windows, as shown in Fig. (2).

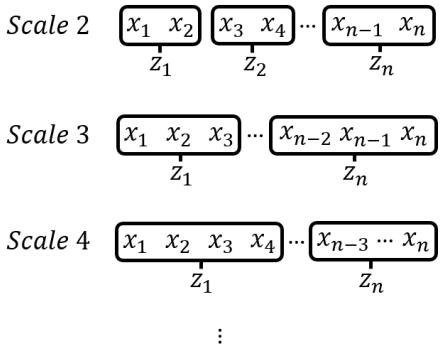

Fig. (2). Construction of coarse-grained time series of scale 2, 3 and 4.

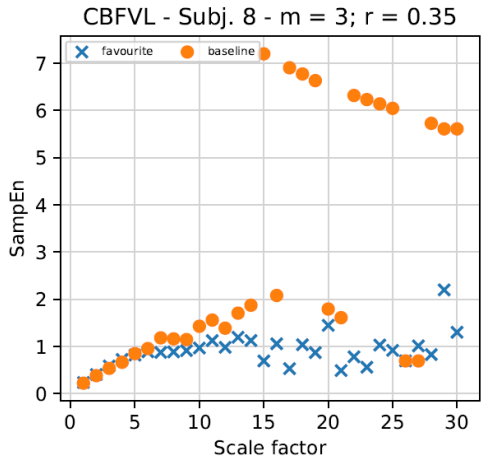

Fig. (3). Entropy signatures obtained using parameters (m=3, r=0.35) and scale factor $1 \leq \tau \leq 30$, calculated for the CBFv signals of Subject 8 recorded while listening to either baseline sound (blue crosses) and the favorite song.

Thus, each element in the coarse-grained time series, $y_j^{(\tau)}$, is calculated according to:

$$y_j^{(\tau)} = 1/\tau \sum_{i=(j-1)\tau+1}^{j\tau} x_i \qquad \text{Eq. (6)}$$

where τ corresponds to the scale factor and $1 \leq j \leq N/\tau$. For scale 1, the coarse-grained time series is the original one.

In the second step, an entropy measure is computed for each of the coarse-grained series and then plotted as a function of the scale factor τ to identify regular patterns and quantify its complexity. This plot, as shown in Fig. (3), is named entropy signature.

Complexity

The integral of the scale-dependent entropy values can be used as a complexity measure [9]. For comparison in this study, the area formed between the values 10 and 30 of the scale factor was utilized as the estimation of the complexity of a signal, as represented in Fig. [4]. The integral calculation was made using Simpson's rule.

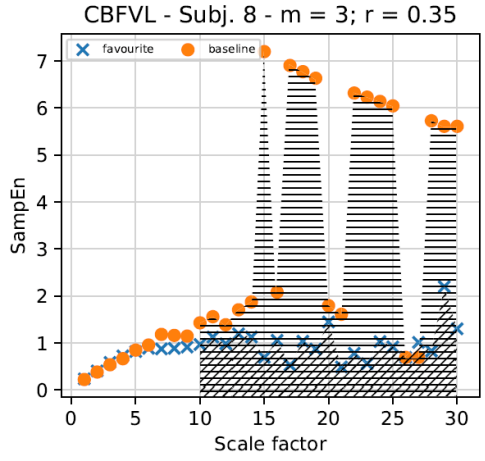

Fig. (4). Complexity estimation of the entropy signatures of Fig. (3). The complexity of favorite song in horizontal lines and the complexity of baseline sound in diagonal lines.

3. SUBJECTS AND MEASUREMENTS

Subjects

Sixteen healthy subjects were recruited for the study in the university campus and hospitals. Research was authorized by the University's Ethics Committee and each participant signed the corresponding written informed consent.

Signals

Non-invasive recordings of CBFv signals were obtained from the bilateral middle cerebral arteries using a DWL Doppler Box system with 2 MHz transducers at a rate of 100 samples per second, while subjects laid supine with their head resting on a pillow. Preprocessing recommendations of Claassen *et al.* [11] were followed and 5 Hz beat-to-beat data were procured for the analysis.

Music Experiment

Volunteers were exposed to five different tasks:

- **Baseline sound**, in which the subjects listened to ambient noise with their eyes open.
- **Silence**, in which the volunteers wore headphones to block any sound.
- **Noise**, in which the participants heard white noise.
- **Arbitrary song**, in which the subjects listened to a randomly selected song without any meaning to them.
- **Favorite song**, in which the volunteers listened to a special song that had emotional positive content for them.

Except for baseline sound, all other tasks were performed with the subjects keeping their eyes closed and wearing good-quality headphones. Each task lasted 7 minutes and were applied in random order. Each task was selected to evaluate different kinds of reactions. Baseline sound is the control stimulus; silence is the simplest task and should not involve much sensory nor emotional brain activity; noise would produce mainly a sensory activation; arbitrary song ought to trigger music-modulated brain activity; and favorite song should add emotion processing.

Parameter Selection

To select the correct range of the scale factor to calculate complexity, two 3-minute-long simulated surrogate tome series were created; using 5% noise low frequency (LF) sinusoidal signals, medium (LFM) at 0.13 Hz and high (LFH) at 0.15 Hz, with an amplitude of approximately 48 cm/s and sampled at 5 Hz. These surrogate signals were analyzed applying MSE with scale factor ranging from 1 to 70 and parameters m = {2, 3} and r = {0.3, 0.35}.

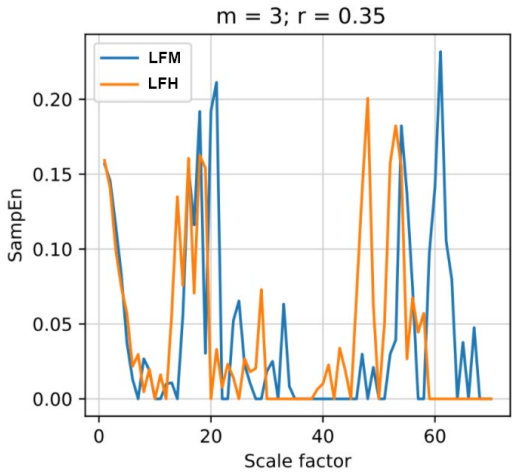

Fig. (5). MSE calculation over two noisy sinusoidal waves to identify higher temporal complexity. Scale factors from 1 up to 70 were considered. Each wave had frequencies of 0.13 Hz (LFM) and 0.15 Hz (LFH).

Following the procedure described in [8], it was determined that the range of scale factor values that reflected temporal dynamical complexity was [10, 30], as seen in Fig. (5).

Statistics

When no significant difference in mean complexity between hemispheres was found (paired comparison), they were averaged for the subsequent analysis.

Repeated-measures ANOVA with Tukey's post-hoc analysis were used; to differentiate mean complexity between the different tasks. Afterward, a receiver operating characteristic curve (ROC) was obtained for the pairs of tasks with higher differences. p-values less than 0.05 were considered statistically significant.

Both Python and R scripts were codified, to apply the MSE analysis and to perform the statistical analysis respectively.

4. EXPERIMENTS AND RESULTS

ANOVA repeated measures over parameters ($m = 3$, $r = 0.35$) got a p-value of 0.01 and Tukey's Post-hoc test a p-value of 0.04 between favorite song and baseline sound. Those differences are summarized in Fig. (6) showing complexity results for the 16 subjects, where baseline sound has the highest mean complexity value (50.07±16.94) in contrast to favorite song having the lower value (34.12±11.71) respectively.

Repeated measures ANOVA over parameters ($m = 3$, $r = 0.35$) resulted significant ($p = 0.010$) and Tukey's post-hoc analysis found a difference ($p = 0.040$) between favorite song and baseline sound. Fig. (6) summarizes the resulting complexity estimations for the 16 subjects, in which baseline sound exhibited the highest mean complexity value (50.07 ± 16.94) in contrast to favorite song that showed the lowest value (34.12 ± 11.71). Using these complexity values to discriminate between signals recorded in either of these two tasks, a ROC curve with an area under the curve (AUC) of 0.785 could be obtained, represented in Fig. (7).

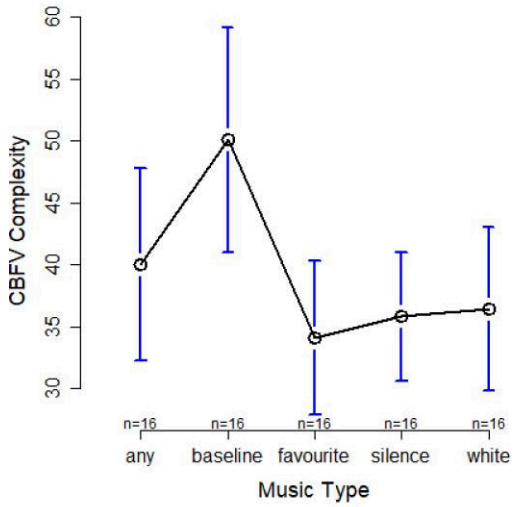

Fig. (6). Mean and confidence intervals of CBFv complexity estimations obtained with parameters ($m = 3$ and $r = 0.35$) in each task: arbitrary song (any), baseline sound (baseline), favorite song (favorite), silence and noise (white).

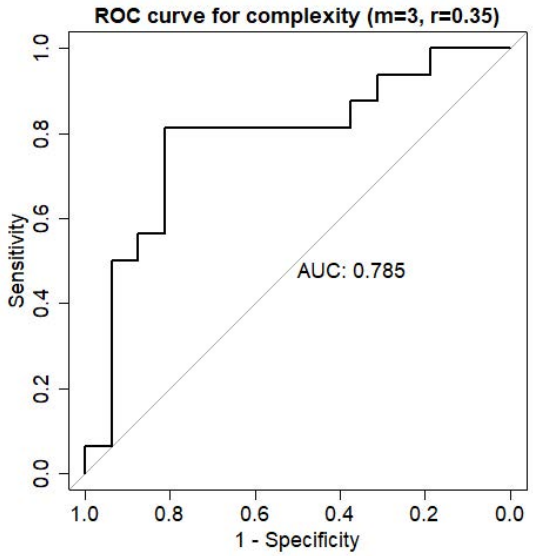

Fig. (7). ROC curve and AUC archived when differentiating baseline sound and favorite song recordings using MSE-derived complexity estimations.

5. DISCUSSIONS

Using the two sinusoidal signals of Fig. (5), whose frequencies are known to be in the range of CBF regulation, between 0.13 Hz and 0.15 Hz, it was possible to determine that they clearly manifested temporal complexity for scale factor valued between 10 and 30. Responses on values after scale factor 30 represent noise. Hence, this range was used to calculate MSE-derived complexity estimations.

Fig. (4) showed an example of an MSE signature for a representative subject, with parameters ($m = 3$ and $r = 0.35$), for the (left hemisphere) CBFv signals recorded during baseline sound and when listening to a favorite song. It can be seen that SampEn grows more rapidly for baseline sound than for the

favorite song. From this plot, a complexity value was gauged for each series as the area under the multiscale SampEn curve [9] in the higher time scales ($10 \leq \tau \leq 30$).

In Fig. (6), the graphical results of the applied repeated measures ANOVA can be seen for the five tasks showing mean and confidence intervals. It is evident that the largest complexity difference exists between baseline sound and favorite song. This difference could be used effectively to discriminate the responses to those tasks as showed in the ROC curve of Fig. (7) that reached an AUC of 0.79.

Moreover, it is interesting to observe that higher complexity values are found when the mind is in a base state and subjects' thoughts manifest freely, in contrast to listening to favorite song, which leads to a decrease in the complexity of the CBFv signal.

These results are consistent with studies that link cognitive charge and cerebral hemodynamics (neurovascular coupling) [12, 13]. Works that studied the relation of music and complexity through EEG also showed associations between music with emotional content and signal complexity [14, 15], although the sense of these connections is not as clear as shown in this study.

6. CONCLUSIONS

Multiscale entropy analysis applied to cerebral hemodynamics is a new research topic with satisfactory results on signal characterization using different music stimuli, and extracting a complexity estimation for each one of them.

Complexity measures derived from MSE signatures showed noticeable classification power when applied to CBFv signals recorded under the different tasks. Larger differences were observed between time series captured at baseline sound and during the playback of a song with emotional content. Moreover, listening to a favorite song yielded the lowest complexity value, which is consistent with the idea that more strenuous cognitive activity might induce some complexity-loss in the cerebral hemodynamics. This opens the possibility of undertaking studies on the action of the mechanisms that control CBF when responding to strong emotions in a wide range of applications.

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Why Are Simple Organisms Haploid and Complex Organisms Diploid?

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ABSTRACT

It is generally known that simple organisms tend to be haploid and complex organisms tend to be diploid. Yet there is no consensus explanation for this phenomenon. This paper attempts to provide an answer. Many studies have found a correlation between species' morphological complexity and the complexity of their protein interaction networks. Other studies have shown that the complex protein interaction networks are controlled by a class of managerial proteins that coordinate and regulate the biological activity within organisms. The managerial proteins must interact with many partners to perform their function, which requires them to be long, to be multi-domain, and to contain intrinsically unstructured regions. Unfortunately, these physical properties make the managerial proteins susceptible to aggregation, which can lead to the accumulation of toxic protein aggregates. Organisms can employ heterozygosity to inhibit protein aggregation, but only if they are diploid. Hence, complex organisms tend to be diploid because they tend to synthesize aggregation-prone proteins. On the other hand, simple organisms tend to be haploid because they synthesize proteins that are less prone to aggregation. The theory may also explain ecological trends associated with organisms of different ploidy level.

Keywords: ploidy level, organism complexity, protein aggregation, protein interaction network, intrinsically unstructured regions, protein length

INTRODUCTION

Organisms vary substantially in the number of chromosomes they possess. A cursory reading of any biology textbook will reveal that simple single-celled organisms tend to possess one set of chromosomes while complex, multicellular organisms tend to possess two sets of chromosomes. In the proper biological jargon, simple species tend to be haploid while complex species tend to be diploid. This phenomenon has important evolutionary implications because diploid organisms can possess two different genetic variants, i.e. they can be heterozygous, whereas haploid organisms cannot. Yet, there is no consensus answer that can explain why simple organisms are haploid and complex organisms are diploid. This paper attempts to provide an answer.

It will be shown that the transition from haploidy to diploidy is direct consequence of the evolution of complexity. In other words, complex organisms must be diploid. This is because complex organisms produce proteins that are prone to misfolding and aggregation. Aggregated proteins are toxic and associated with many diseases, thus all organisms have evolved various protein maintenance systems to keep aggregated proteins from accumulating within their cells. Heterozygosity can be used to slow protein aggregation reactions, but an organism can only be heterozygous if it is diploid. Therefore, complex organisms need to be diploid to keep their protein maintenance burden under control. Why do complex organisms produce aggregation-prone proteins, and how does heterozygosity slow protein aggregation reactions? The rest of this paper will answer those two questions.

PROTEIN INTERACTION NETWORKS AND COMPLEXITY

Complex organisms need complex protein interaction networks to coordinate and regulate all their biological activity (Rubin *et al.*, 2000; Patthy, 2003; Tan *et al.*, 2005; Stumpf *et al.*, 2008; Xia *et al.*, 2008). The complex protein interaction networks, in turn, require a class of managerial proteins that have numerous interaction partners. Included among the ranks of these managerial proteins are transcription factors, kinases, proteases, and phosphatases (LeMosy *et al.* 2001; Walhout 2006; Lopez-Otin and Hunter 2010; Wang and Chen 2010; Cheng *et al.* 2011). Managerial proteins are responsible for the regulation and signaling necessary to synchronize the various biochemical pathways operating in an organism. Unsurprisingly, complex organisms produce more of these kinds of proteins (Patthy, 2003; Tordai *et al.*, 2005; Ekman *et al.* 2007).

Managerial proteins tend to be large and possess multiple domains (Tan *et al.*, 2005; Tordai *et al.*, 2005; Ekman *et al.*, 2007). A domain is a sequence of amino acids that, if separated from the rest of the polypeptide chain, would still fold into its proper conformation and function as a normal protein. A multi-domain protein can be considered as a string of joined proteins. In fact, proteins that exist separately in some species may be found as parts of multi-domain proteins in other species, a phenomenon called domain accretion (Koonin *et al.*, 2002; Basu *et al.*, 2009). Proteins with a lot of interaction partners tend to be multi-domain because multiple domains tend to facilitate interactions with different partners (Tan *et al.*, 2005; Tordai *et al.*, 2005; Ekman *et al.*, 2007). Multi-domain proteins are typically longer than single domain proteins simply because they contain more domains. In fact, there is a linear correlation between the number of interactions partners a protein has and its length (Tan *et al.*, 2005).

Managerial proteins also tend to possess intrinsically unstructured regions (IURs). IURs are common in multi-domain proteins because they server as interdomain linkers that allow each domain to move independently of the others (Tompa, 2002; Tompa, 2005). IURs also occur near binding sites, which allow the binding sites to assume multiple conformations that can bind to different partners (Dunker *et al.* 2005; Uversky and Dunker 2010). Thus, IURs are common in proteins that have many interaction partners (Olszcha *et al.,* 2011; Uversky, 2019). In fact, 94% of transcription factors and 70% of signaling proteins in eukaryotes contain IURs (Iakoucheva *et al.* 2002; Liu *et al.* 2006; Uversky and Dunker 2010).

In summary, complex organisms require complex protein interactions networks to coordinate and regulate their biological processes. The managerial proteins that control these protein interaction networks tend to be long, tend to be multi-domain, and tend to contain IURs. Unfortunately, these physical properties tend to make the managerial proteins more prone to aggregation, which requires complex organisms to utilize various maintenance systems to keep protein aggregation under control.

PROTEIN AGGREGATION AND MAINTENANCE

Olszcha *et al.* (2011) designed an assay to identify aggregation-prone human proteins and found that they were both large and typically contained intrinsically unstructured regions (IURs). Unfortunately, these properties are associated with the managerial proteins that underly the sophisticated protein interaction networks in complex organisms. Thus, it is useful to understand why proteins aggregate and how organisms cope with this problem.

Proteins consist of sequential chains of amino acids. To function properly, the chains must fold into the proteins' proper conformations. However, sometimes proteins misfold, exposing binding sites that allow two separate proteins to bind together, which results in protein aggregation (Silow and Oliveberg 1997; Bitan et al. 2001; Kayed et al. 2004; Cleary et al. 2005; Haass and Selkoe 2007; Vieira et al. 2007). Protein aggregates are toxic, and organisms must remove them in order to stay alive (Kayed et al. 2003; Haass and Selkoe 2007; Vieira et al. 2007; Shankar et al. 2008).

Organisms have evolved sophisticated protein maintenance systems to prevent the accumulation of protein aggregates (Hartl and Hayer-Hartl, 2002; Albanese *et al.*, 2005). These maintenance systems make use of proteins called chaperones that bind to misfolded and aggregated proteins and initiate either their repair or destruction. Rebeaud *et al.* (2020) has shown that these maintenance systems are more elaborate in complex organisms than simple organisms. For example, the number of chaperone genes increased 30-fold from simple algae to complex plants, and the abundance of chaperones in the cytosol of cells increased 6-fold from bacteria to mammals. Furthermore, only eukaryotic cells contain aggresomes, which are membrane-bound garbage collection sites for aggregated proteins (Kopito, 2000). Complex organisms have had to evolve other methods to keep protein aggregation under control, which will be described below.

PROTEIN LENGTH AND ORGANISM COMPLEXITY

The length of a polypeptide chain should affect the competition between its folding and aggregation reactions. Since there is some degree of randomness associated with the folding process and longer polypeptide chains have to establish more contacts than shorter chains, longer polypeptide chains are expected to take longer to complete folding than shorter proteins (e.g. Ivanokov *et al.*, 2003). On the other hand, longer polypeptide chains have a greater potential for aggregation when they approach each other because aggregation can occur at more sites along the unfolded polypeptide chain (e.g. Anderson *et al.*, 2010). Consequently, longer polypeptide chains should be more prone to aggregation and have a lower folding efficiency than shorter polypeptide chains.

The median length of eukaryote proteins is 50% longer than the median length of prokaryote proteins (Brocchieri and Karlin, 2005). About 65% of prokaryote proteins are multi-domain whereas 80% of eukaryote proteins are multi-domain (Apic *et al.*, 2001). This creates a greater protein misfolding and aggregation burden for eukaryotic species than prokaryotic species as evidenced by the poor yields of recombinant eukaryotic proteins expressed in prokaryotes (Siller et al. 2010).

Netzer and Hartl (1997) proposed that eukaryotes compensate for their greater protein misfolding/aggregation burden by translating proteins at a much slower rate than prokaryotes. For example, *E. coli* typically translate polypeptide chains at a rate of 10-20 amino acids per second whereas eukaryotes typically translate proteins at a rate of 3-8 amino acids per second. Since proteins begin to fold before they are completely synthesized, a slower rate of translation will allow more folding to occur before the nascent polypeptide chain is released from its ribosome. Thus, they argue, eukaryotes need to translate proteins at slower rates than prokaryotes because their proteins are longer, more susceptible to aggregation, and need more time for co-translational folding.

Siller *et al.* (2010) provide experimental support for the ideas presented in Netzer and Hartl (1997). Using a mutant strain of *E. coli* that translates proteins at rates typical of eukaryotes, they examined the extent of aggregation of recombinant proteins taken from *Saccharomyces cerevisae*. Extensive protein aggregation occurred in *E. coli* strains that synthesized the recombinant proteins at wild-type rates, but much less protein aggregation occurred in the *E. coli* strains that synthesized the recombinant proteins at rates typical of eukaryotes. They conclude that *S. cerevisae* proteins fold more slowly than *E. coli* proteins and are more prone to aggregation, thus necessitating the need for slower translation rates.

So far, we have compared the protein lengths of single-celled prokaryotes and eukaryotes. Several studies have shown a correlation between organism complexity and protein complexity across the entire spectrum of organism complexity, from bacteria to animals (Tordai *et al.*, 2005; Ekman *et al.*, 2007; Rebeaud *et al.*, 2020). In general, organisms with greater morphological complexity have a higher proportion of multi-domain proteins.

Using data from the UniProt Knowledgebase, Tordai *et al.* (2005) created databases of proteins synthesized by 2 bacteria, 3 archaea, 1 protist, 1 plant, 2 fungi, and 3 animals. They estimated the relative number of multi-domain proteins in each of these groups by determining the percentage of proteins that had two or more domains annotated in the Pfam-A database. The results, shown in Table 1, are that the proportion of multi-domain proteins decrease in order of animals > plants > fungi ~ protist > bacteria > archaea. 39% of animal proteins contain two or more Pfam-A domains while only 23% of archaea proteins have two or more Pfam-A domains.

Ekman *et al.* (2007) shows a similar link between morphological complexity and protein complexity. They define the sequence of domains in a protein as its domain architecture (DA). If the sequence is only one domain, then the protein has a single domain architecture (SDA). If the sequence contains multiple domains, then the protein has a multi-domain architecture (MDA). Ekman *et al.* (2007) created databases of proteins from 7 animals, 2 plants, 2 fungi, 7 bacteria, and 7 archaea and determined the percentage of each groups' domain architecture that are MDAs. The results, shown in Table 1, are that the proportion of MDAs decrease in order of animals > plants > fungi > bacteria ~ archaea. Thus, the results of Ekman *et al.* (2007) agree with Tordai *et al.* (2005).

Rebeaud *et al.* (2020) provides the most recent analysis of protein complexity across the spectrum of organism complexity and corroborates the earlier studies. They found the median protein length produced by a species has increase 3-fold from bacteria to mammals. The number of multi-domain proteins produced by a species has increased 300-fold across the same complexity range.

TABLE 1

Tordai et al., 2005		Ekman et al., 2007		
Category	Multidomain proteins (% of proteins)	Category	Multidomain architectures (% of domain architecture)	Species
Bacteria	27%	Animals	49%	*Homo sapiens*
Archaea	23%		49%	*Mus musculus*
Protozoa	32%		49%	*Rattus norvegicus*
Plants	35%		49%	*Gallus gallus*
Fungi	32%		49%	*Danio rerio*
Metazoa	39%		44%	*Drosophila melanogaster*
			44%	*Caenorhabditis elegans*
		Plants	37%	*Arabidopsis thaliana*
			35%	*Oryza sativa*
		Fungi	33%	*Saccharomyces cerevisiae*
			33%	*Schizosaccharomyces pombe*
		Eukarya	53%	
		Bacteria	29%	
		Archaea	29%	

Table 1. The data on the left is from Tordai et al. (2005) and shows the percentage of proteins that are multi-domain within each of the paper's categories. The data on the right is from Ekman et al. (2007) and shows the percentage of domain architectures that are multi-domain architectures for each species.

The data clearly shows an acceleration in protein length and multi-domain protein frequency among eukaryotic lineages than prokaryotic lineages.

INTINSICALLY UNSTRUCTURED REGIONS AND ORGANISM COMPLEXITY

Olszcha *et al.* (2011) designed an assay to identify aggregation-prone human proteins and found that they were both large and typically contained intrinsically unstructured regions (IURs). Unfortunately, proteins that contain IURs also tend to be metastable and more prone to aggregation (Olszcha *et al.,* 2011; Uversky, 2019). This is because IURs give proteins the flexibility to properly align their backbones and form the cross-β interactions that bind the proteins together (Carrio *et al.* 2005; Nelson *et al.* 2005; Ventura 2005; Wang *et al.* 2008; Ramshini *et al.* 2011; Stroud *et al.* 2012). However, aggregation-prone proteins are typically not completely disordered, but only contain disordered regions, leading some authors to call them semi-disordered (Zhang *et al.*, 2013 and Uversky, 2019).

Complex organisms tend to produce more proteins containing IURs. Ward et al. (2004) found the relative abundance of proteins containing IURs are 2%, 4%, and 33% for archaea, bacteria, and eukaryotes, respectively. Iakoucheva *et al.* (2002) estimates that approximately, 50% of mammalian proteins contain IURs. Niklas et al. (2018) has shown a positive correlation between number of cell types and the number of IURs found in proteins of different species across algae, plants, and animals. The relationship does not appear to be linear, however, because Xue *et al.* (2012) found a clear correlation between cell type number and percentage of proteins containing IURs for species that possess 20-40 cell types, but no correlation for species that possess more than 40 cell types. Perhaps there was no need for more IURs once the foundations for multicellularity had been laid.

SIMPLE ORGANISMS ARE HAPLOID. COMPLEX ORGANISMS ARE DIPLOID. WHY?

Organisms can use heterozygosity to prevent the accumulation of protein aggregates (Ginn 2010, Ginn 2017). The process of protein aggregation is highly specific in that protein aggregates are highly enriched with a single protein species (London *et al.*, 1974; Speed *et al.*, 1996; Kopito, 2000; Rajan *et al.*, 2001; Ventura, 2005; Morell *et al.*, 2008). Several authors have shown that a single point mutation is enough to inhibit the coaggregation of protein polymorphisms (Mead *et al.*, 2003; O'Nuallain *et al.*, 2004; Apostol *et al.*, 2010; Ganesan *et al.*, 2014; Betti et al., 2016). Thus, an organism that is heterozygous at a gene locus can produce two different polymorphisms of a protein that do not co-aggregate, thereby lowering the organism's total protein maintenance burden. A similar process has been shown to inhibit the aggregation of the prion that causes Kuru disease (Palmer, 1991; Mead, 2003).

A little arithmetic can illustrate why heterozygosity lowers protein aggregation rates. Two protein molecules must collide before an aggregation reaction can occur. If an organism is heterozygous at a protein-coding gene locus, then it will produce two different polymorphisms of a protein. In any time interval, 50% of the protein collisions will be between identical polymorphisms and 50% of the collisions will be between different polymorphisms. In contrast, an organism that is homozygous at the same gene locus can only produce one

polymorphism of the protein, and all the protein collisions must be between that single polymorphism. Thus, protein aggregation reactions tend to proceed more slowly in organisms that are heterozygous at protein-coding gene loci than in organisms that are homozygous at protein-coding loci. Adding up this inhibition effect over many gene loci can lead to an appreciable reduction in an organism's overall protein maintenance burden.

It is now possible to explain why simple organisms are haploid and complex organisms are diploid. Complex organisms produce more proteins that are prone to aggregation than simple organisms. Therefore, complex organisms derive more of a benefit from being heterozygous than simple organisms. Since an organism cannot be heterozygous if it is haploid, complex organisms have increased their ploidy level so they can use heterozygosity to help keep their protein maintenance burden under control.

Support for this theory comes from the relative stress tolerances of simple and complex organisms. Many studies have observed that animals are more sensitive to extreme environments that promote protein aggregation than less complex organisms (Alpert, 2006; Kranner *et al.*, 2008). Nearly all thermophiles are prokaryotes (Koonin *et al.* 2002; Brocchieri and Karlin, 2005). In contrast, animals typically exhibit much greater sensitivity to thermal stress (Portner, 2001; Dilly *et al.*, 2012). Bacteria and archaea are capable of growing at extremely high salinities (Kunte *et al.*, 2002), withstand desiccation (Potts, 1994; Alpert, 2006), or grow in freezing conditions (Russell, 1998). Not all prokaryotes can grow in extreme environments because specific adaptations, such as those promoting membrane integrity and DNA stability, are required (Konings *et al.* 2002; Trivedi *et al.* 2005). However, prokaryotes may be pre-adapted to these environments because their small proteins are less aggregation prone and, therefore, more resilient. In contrast, the ability to withstand such stresses is more limited in animals, tardigrades and bdelloid rotifers being notable exceptions (Alpert, 2006).

Plants are particularly useful for illustrating the theory because they alternate between haploid gametophyte generations and diploid sporophyte generations. However, the dominant generation varies between species, which can be divided into three categories: bryophytes, ferns, and spermatophytes. Bryophytes (mostly mosses) have a dominant gametophyte generation that reproduces sexually to produce a short-lived sporophyte. The sporophyte grows out of the gametophyte parent and depends on it for sustenance. The sporophyte produces haploid spores that germinate to form new gametophyte plants. Ferns have gametophytes and sporophytes that can exist independently of each other. In ferns, the sporophyte grows out of the gametophyte parent, but is self-sustaining. In fact, the gametophyte typically dies shortly after fertilization. Spermatophytes (seed plants) have a dominant sporophyte generation that encompasses almost the entire lifespan of the organism. The gametophyte generation is retained only in the gametes. Thus, plants may sit on the boundary of the haploid-diploid transition with bryophytes on one side of the boundary, ferns sitting on the boundary, and spermatophytes on the other side of the boundary.

Once again, there is a relationship between the complexity of the species and its ploidy level. The primarily haploid bryophytes are relatively simple plants, typically 2 cm tall and one cell thick. The primarily diploid spermatophytes are the most complex of plant species and include all flowering plants. The ferns alternate between a haploid generation that is simple, resembling a bryophyte, and a diploid generation that is significantly larger and more complex. The studies of Tordai *et al.* (2005), Ekman *et al.* (2007), and Rebeaud *et al.* (2020) show that the percentage of multi-domain proteins in plants is the second highest among their categories of life, slightly higher than fungi. However, their studies only included angiosperms and not bryophytes or ferns. This provides an opportunity for a prediction. The theory presented in this paper predicts that the bryophytes should have a smaller percentage of multi-domain proteins (comparable to the percentage seen in fungi) than spermatophytes.

The relative stress tolerances of the different plant divisions provide support for the hypothesis that spermatophytes synthesize longer proteins than bryophytes. Organisms that produce short proteins should be pre-adapted to environments that promote protein aggregation, enabling them to readily migrate into and adapt to such environments. In contrast, organisms that produce aggregation-prone proteins should struggle to survive in harsh environments. Bryophytes are much more tolerant of freezing, desiccation, salinity, and thermal stresses than spermatophytes (Alpert, 2000; Oliver *et al.*, 2005; Wang *et al.*, 2009; Smale *et al.*, 2017). They can be found in extremely cold, hot, and arid environments not inhabited by more complex plants (Longton, 1988; Alpert, 2006; Procter and Tuba, 2002; Kranner *et al.*, 2008; Smale *et al.*, 2017). Fern stress tolerance is a little more complicated. Fern sporophytes show limited desiccation tolerance and are comparable to spermatophytes, but fern gametophytes show greater levels of desiccation tolerance and are comparable to bryophytes (Watkins *et al.*, 2007; Hietz, 2010). In fact, some tropical fern species have loss the sporophyte stage of their life cycle and now propagate as asexually reproducing gametophytes, which allows them to live in colder and drier habitats than their sporophyte-producing relatives (Farrar, 1978; Farrar, 1990). Thus, the relative order of tolerance to stresses that promote protein aggregation is bryophytes ~ fern gametophyte > spermatophyte ~ fern sporophyte. This trend corresponds to the relative complexity of the plants and the expected protein complexity within each division.

CONCLUSION

This paper has proposed a theory to explain why simple organisms are haploid and complex organisms are diploid. The outline of the theory is straightforward. Complex organisms need complex protein interaction networks to coordinate and regulate all of their biological activity. The protein interaction networks, in turn, require a class of managerial proteins that have a lot of interaction partners. The managerial proteins tend to be long and multi-domain because multiple domains are needed to facilitate multiple interactions. The managerial proteins also tend to contain intrinsically unstructured regions which enable them to assume multiple conformations that can interact with different partners. Unfortunately, these properties make the managerial proteins aggregation prone. Complex organisms have several tools for keeping protein aggregation under control. One of those tools is heterozygosity since heterozygous organisms can produce different protein polymorphisms that do not co-aggregate. However, complex organisms can only use heterozygosity to keep protein aggregation under control if they are diploid. Therefore, as some evolutionary lineages have gotten more complex over time, at some point they had to switch from being haploid to being diploid.

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Apply Explainable AI to Sustain the Assessment of Learning Effectiveness

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ABSTRACT

With the rapid development of internet and digital technologies, online learning is becoming prevalence, and the online education market has shown significant growth over the past decade. Nowadays, online learning is probably the most essential and used option for educational institutions all over the world to conduct their teaching and learning because of the impact of the COVID-19 pandemic. Most of these institutions have implemented various learning management systems (LMS) to facilitate the delivery and measurement of their online learning programs. Since the LMS systems keep recording and tracking the fulfillment of lectures, assignments and examinations about learners and function as a big data repository, the educational data mining (EDM) could explore and analyze the learning effectiveness of learners based on their involvement in learning and teaching.

In general, sophisticated machine learning (ML) and artificial intelligence (AI) technologies provide solutions to understand the behavior of learners from their personalized learning profile. However, the pervasive deep learning techniques developed for AI come from the original neural network, and their network structure and interconnected weights are considered as a black box that is hard to be interpreted by humans. To enable the deep learning techniques to provide meaningful insight about learner's outcomes, this study developed an explainable artificial intelligence (XAI) technique to analyze the relationship between learning inputs and corresponding achievement for each learner. The proposed XAI approach applied an innovative method of model grafting to integrate the traditional machine learning with deep learning models. The grafting method used the decision tree as a global model to provide interpretable ability and then concatenated with the local models, which could choose the deep neural network or K-nearest neighbor model.

The global model was considered as the primary model to provide human-readable decision rules and variable importance, while the local model might be used to improve prediction accuracy or provide additional interpretability. As the local model could be implanted to different branches of the global model, a fusion mechanism was designed to merge the variable importance from both models for each learner. The outcome of the hybrid models might identify important factors that affect learning progress and even predict the risk of a learner failing a subject based on providing interpretable decision rules.

Keywords: Model Grafting, Explainable AI, Model Fusion, Educational Data Mining

1. INTRODUCTION

As both classroom teaching and online learning environment such as large-scale open online courses (MOOC) generate a large amount of data related to the learning and teaching process, this valuable information could be analyzed by data mining techniques to improve the learning effectiveness of students. In addition, the rapid development of artificial intelligence in recent years has also added many practical algorithms and analysis tools to educational data mining (EDM) and learning analytics (LA). The EDM usually uses different methodologies to integrate machine learning, statistical tools and database systems to extract useful information from large databases to generate new insights about learning effectiveness of students. At present, both EDM and LA are emerging research fields, and they are committed to developing innovative analysis methods and techniques to explore and understand the huge educational learning data obtained from the educational environment.

The educational data mining generally uses artificial intelligence (AI), machine learning (ML), and statistics-based algorithms to construct analysis models and emphasize the use of automated methods to build models in educational data analysis. On the other hand, the learning analytics is the use of measuring, collecting, analyzing and reporting on the information generated by learners during their learning process. The purpose of the EDM and LA are to understand the learning activities of students track their learning progress, evaluate their academic performance, and most of all, to detect who are at risk of failure.

The applications of these two research fields are well described in the study [1]. The authors analyze 402 articles related to the application of educational data mining and learning analytics in higher education, and the research results find that both of them can indeed be used to solve various specific learning problems and provide the best solutions. Another study [2] also states the application of both fields and compares their differences, similarities, and challenges in more details. In another work [3], an educational data mining study is conducted in Europe and Australia through the use of association rules and fuzzy sets in data mining technology. The study suggests that association rules are an effective method to understand the effectiveness of learning activities related to learners. In addition, the research work in [4] uses different data mining algorithms to carry out predictive analysis for the academic performance of students in the first three years, and the results are used to screen out students who have poor academic performance and provide early warning and guidance.

Since most of our courses use blended learning, which combines online learning with classroom teaching, an extensive amount of data is generated and stored in the learning management systems (LMS). This research work tries to integrate AI and ML technologies to evaluate the learning effectiveness of students based on the data of the student involvement in learning and teaching. The analysis is conducted in the learning process to search for the students suffered from learning difficulty, and provides solutions to improve their academic performance. In general, sophisticated machine learning (ML) and artificial intelligence (AI) technologies provide solutions to understand the behavior of learners from their personalized learning profile. Moreover, the latest development of explainable artificial

intelligence techniques (XAI) in recent years is used to analyze the learning process of individual students, evaluate important factors that affect the student failure.

2. RESEARCH FRAMEWORK AND METHODOLOGY

Although many academic institutions use various automated data mining systems currently based on artificial intelligence in applications such as online learning, assessment and other activities, the instructors might be unable to know how the algorithms work behind the systems even they produce high efficiency and accuracy results. However, the network structure and weights derived from the deep learning techniques are usually considered as black-box operations. Therefore, their analysis and decision results might be hard for the human to understand whether the results are reasonable. In order to avoid the irreparable harm caused by the automated systems, the European Union implemented the General Data Protection Regulation (GDPR) in 2018, which suggests that the applicants have the right to know the reasons leading to the decisions been made to them. Consequently, for the application of educational mining cases, decision makers might need to provide reasons for their decisions. As this study aims to construct a teaching support system based on ML and AI algorithms to predict student performance and identify whether the students suffer from learning difficulty or at risk of failing a course, the system needs to be capable of providing interpretability.

The proposed system provides the complementary analysis results of both global and local models to predict the learning effectiveness of students based on the LMS data recording the student involvement in learning and teaching. Moreover, the explainable artificial intelligence techniques (XAI) developed in recent years is also applied to analyze the learning process of an individual student, evaluate important factors that affect learning effectiveness, and derive decision rules to predict student learning outcomes. The dedicated hybrid XAI approach allowed users to select a desired tradeoff between the accuracy and interpretability for the model. Normally, as the accuracy of a model increases, the model becomes more complexity in exchange for the cost of interpretability.

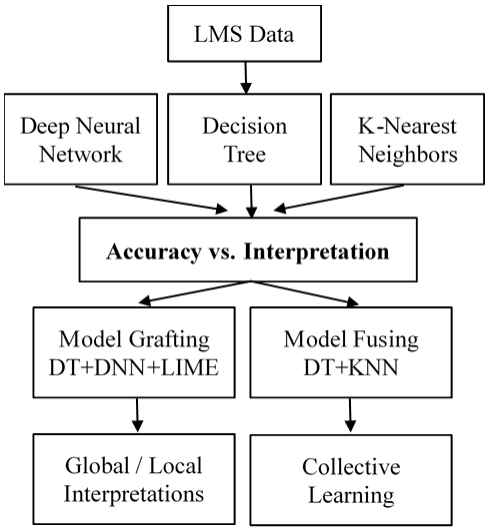

Figure 1. The Research Framework

As shown in Figure 1, the research framework for the development and implementation of the integrated system is an iterative processing approach that includes three stages to conduct the performance assessment of students in teaching practice. The first stage uses three machine learning models including decision tree (DT), deep neural network (DNN) and K-nearest neighbors (KNN) to predict students' learning effectiveness based on their online activities and academic results. The second stage uses a model grafting technique that employs the DT model as a global model and supplements it with a local DNN model. The resulting hybrid XAI model allows users to acquire a balance between the accuracy and interpretability as they wish. In the third stage, the model fusion technique is used to integrate the DT model with a KNN model to complement each other. The synergy of both models could help to identify the students at risk of failing a course and reduce the type I and II errors of the predictive models. All three stages could proceed iteratively several times during the whole semester.

2.1 Global and Local Interpretations

Although the AI and ML systems could supplement human decisions by identifying meaningful patterns from data and learning from experiences, the commercial AI applications are subject to regulatory compliance that might be regularly revisited to meet the law's requirements. For example, the Dodd-Frank Wall Street Reform and Consumer Protection Act (Dodd-Frank Act), the General Data Protection Regulation (GDPR), the Economic Growth, Regulatory Relief, and Consumer Protection Act (EGRRCPA) require transparency and explainability in the decision-making process. Therefore, the AI and ML applications might need explainability of models, including both global and local interpretation, to ensure compliance with regulations. Basically, global interpretations attempt to understand the input variables and their entire modeled relationship with the output variables, whereas the local interpretations try to model predictions for a single sample based on a group of similar regional samples.

In contrast to the DT model often cited as interpretable model, the KNN and DNN models are employed as a counterpart to examine whether the non-tree-based models could improve the system performance. The DNN model is applied not only as a global model to compare their performance but also as a local model in conjunction with the DT global model to construct a hybrid system, even though its intrinsic characteristics of black box is difficult for humans to comprehend. To make the DNN model interpretable for users, the explainable AI (XAI) techniques [5] are required to provide explanations that are compatible with their original models.

When using AI models for prediction purpose, it is difficult to balance the interpretability and the predictive accuracy of the model since the need for explanations varies and depends on the applied domains. In general, Partial Dependence Plots (PDP), Local Interpretable Model-Agnostic Explanations (LIME) and Shapley values (SHAP) are all well-known explanation techniques applied to ML models. The PDP model [6] changes the value of a certain characteristic variable one by one while controlling other variables and interprets the relationship between the characteristic variable and the target variable by a line graph. Comparatively, the Shapely explanations method applies the Shapley value designed by Lloyd Shapley [7] to explain the importance of feature variables. The LIME algorithm uses a small number of regional samples to construct a simple local model as a proxy model to interpret the original black-box model [8]. Since the LIME algorithm is model-agnostic, the

global model can be any machine learning or deep learning algorithm, while the local model can apply a simple linear regression to fit the target values predicted by the original global model.

2.2 Model Grafting Method

To reduce the tradeoff between accuracy and interpretability in machine learning, a practical model grafting method in our previous work [9-10] is proposed to combine the global model with a local model. The DT model is applied as the global model due to its explainable ability, and the DNN and KNN models could be chosen as a local model where the former intends to increase the accuracy and the latter attempts to increase the interpretability by seeking students with the similar risk of failing a course.

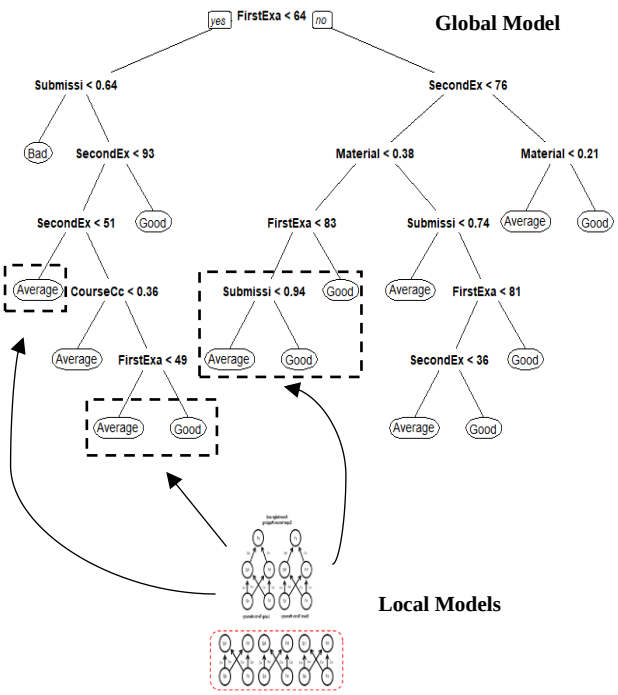

Figure 2. Global and Local Models Grafting

As shown in Fig. 2, the model grafting performs on the terminal nodes of a decision tree, and the primary structure of the original decision tree remains unchanged. The terminal node grafting method could control the interpretability of the decision tree by modifying the tree depth and branches, and the model accuracy could be improved depending on what kind of local model been applied.

The research work [11] has demonstrated that the combination of both pruning and grafting might effectively provide the best general predictive accuracy. Although most of the grafting approaches append new branches with another decision tree to increase accuracy, the DNN models are used as the local model in this study rather than using other non-tree-based model. Both the terminal node grafting and the internal node grafting could be developed to improve the accuracy and interpretability of the hybrid model. As the DNN model employs a black-box algorithm, the model could be interpreted by the LIME model that applies a simple lasso or ridge regression, or another decision tree to increase its interpretability. Since the LIME model is a surrogate model trained to approximate the predictions of the DNN model, it does not explain the DNN model globally and might not satisfy the ECOA regulations for application in the credit risk industry. However, the LIME model is limited to provide the important variables for the local models in our educational data mining, while the DT model is the primary model to explain the risk rating of learners globally. Based on the model grafting, the global model affiliated with its local models could help to provide more explainable information, such as the evaluation of the major factors that cause student failure and related decision rules.

2.3 Model Fusion Method

Unlike the model grafting method concatenating the global model with local models, the model fusion method requires both DT and KNN models to coexist as a global model, with each one complementing the other. As the ECOA regulations requests "The reasons disclosed must relate only to those factors actually scored in the system," which might be applicable to the educational data mining in the future, both the DT and KNN models are fusion to generate prediction result directly without the need of a proxy model such as LIME model to provide explainability. In this study, the students achieve good to excellent performance and receive a final grade above 80% are classed as "Good". On the other hand, the students demonstrate generally weak to satisfactory performance and receive a final grade between 60% and 80% are classed as "Average". If the students receive a final grade below 60%, they will be regarded as unacceptable performance and classed as "Bad".

Table 1. Confusion Matrix (DT Model)

		Actual Rating		
		Bad	Average	Good
Predicted Rating	Bad	**30**	**5**	0
	Average	**34**	224	37
	Good	1	71	326

The training outcome of a DT model creates the confusion matrix like the Table 1, both the false positive (FP) and false negative (FN) for the "Bad" class is our major concerns, because these students achieve marginal performance and are at higher risk of academic failure even they have been incorrectly predicted as "Average" class. All observations classified as false positives, false negatives and true positives in "Bad" class are collected and appended to the neighborhood groups for training KNN model. Our model fusion is a collective learning process that the KNN model expands its neighborhood groups for searching the best K neighbors through new classified cases been predicted by the other DT model. The synergy results of both models might help to discover the actual "Bad" student been classified as "Average" and vice versa due to the type I and II errors.

3. EXPERIMENT RESULTS

The experimental data collected from the LMS system contain 1040 anonymized student across different study-level regarding their online activities and academic results from 2016 to 2019. The dataset consists of 16 variables and is partitioned to 70% for training the model and another 30% for evaluating the accuracy

of the model. Continuous variables are normalized and categorical variables are encoded according to the requirement of the individual model. The metrics derived from the confusion matrix including Accuracy, Kappa, Sensitivity and Specificity are used to measure the model performance. The experimental results for the three stages are summarized in the following sections.

3.1 Experimental Results for the First Stage

In the first stage, the DT, DNN and KNN models were employed as the single global model to predict the risk of student failure. The DNN model was constructed with five different layers, including a fully connected last layer, and the dropout layers with a cutoff value of 0.1 were used to reduce the overfitting problem. The activation functions such as Sigmoid, Tanh and ReLU were assessed in different hidden layers and another Softmax function was used in the last fully connected layer. The DT model was pruned with the best complexity parameter (CP), which controlled the number of splits in a decision tree by examining the misclassification error for each branch and was evaluated between 0.01 and 0.001.

Table 2. Performance of the DT model (accuracy and kappa)

Models	Accuracy	Kappa
DT(Training)	0.797	0.634
DT(Testing)	0.734	0.536

Table 3. Performance of the DT model (other metrics)

models	Metrics	Bad	Average	Good
DT(Training)	Sen.	0.548	0.669	0.827
	Spe.	0.971	0.791	0.770
DT(Testing)	Sen.	0.461	0.746	0.898
	Spe.	0.992	0.834	0.802

Table 4. Performance of the all models (accuracy and kappa)

Models	Accuracy	Kappa
DT	0.734	0.536
KNN	0.772	0.596
DNN	0.814	0.676
DT-DNN	0.785	0.630

As shown in Table 2 and 3, the experiment result reported that the DT model performed an accuracy of 0.734 for testing, about 6.3% difference from its training accuracy. Although the accuracy of DT model was slightly lower than other models, the DT model was selected as the primary global model to concatenate with other local models due to its interpretable ability. The results indicated that the DNN model achieved the highest accuracy of 0.814, where 72% of the failing students were correctly identified.

For an early warning system, the sensitivity was more important than specificity because the "Bad" class was classified as "Average". However, the sensitivity of DT model was only 0.461, which might require some further improvement. On the other hand, the DNN model achieved the highest sensitivity of 0.720 among three models, but the model suffers from its poor explainable ability. The KNN models were evaluated with two different distance measures, including the Euclidean distance and Minkowski distance. The experiment results for all three ML models were shown in Table 4 and 5.

As the global model was the primary model to provide explainable ability to help people understand its outcomes. The DT model was selected as global models because of its interpretable decision rules and the ability to compare variables importance. The tree structure of the DT model is converted to a set of if-then decision rules as the excerpted rules illustrated in Table 6.

Table 5. Performance of the all models (other metrics)

Models	Metrics	Bad	Average	Good
DT	Sen.	0.461	0.746	0.898
	Spe.	0.992	0.834	0.802
KNN	Sen.	0.484	0.723	0.874
	Spe.	0.996	0.813	0.776
DNN	Sen.	0.720	0.792	0.846
	Spe.	0.955	0.834	0.907
DT-DNN	Sen.	0.754	0.677	0.834
	Spe.	0.819	0.972	0.839

Abbreviations: Sen. (%) (Sensitivity), Spe. (%) (Specificity)

Table 6. The Excerpted Decision Rules of the DT Model

No.	Description of Decision Rule
2	FirstExam < 63.5 & SubmissionPct>= 0.635 & SecondExam < 51 => Average
6	FirstExam < 63.5 & SubmissionPct>= 0.635 & SecondExam >= 92.5 => Good
10	FirstExam >= 63.5 & SecondExam < 75.5 & MaterialViewPct >= 0.375 & SubmissionPct< 0.735 => Average
11	FirstExam >= 63.5 & SecondExam < 75.5 & MaterialViewedPct >= 0.375 & SubmissionPct>= 0.735 & FirstExam < 81 & SecondExam < 36 => Average
15	FirstExam >= 63.5 & SecondExam >= 75.5 & MaterialViewedPct >= 0.205 => Good

3.2 Experimental Results for the Second Stage

The experiment results of the second stage showed that the accuracy of the predictions made by the DT-DNN model increased from 0.743 to 0.785 if compared to the original DT model without grafting with the local model. The results also indicated 75.4% of the failing students were correctly identified as "Bad" class. Although this grafting approach was unable to outperform the DNN model in predictive accuracy, the sensitivity improved by 3.4%. This result suggested that the actual "Bad" students misclassified as the "Average" and "Good" classes decreased. The DNN model could help to improve the accuracy without losing the interpretability if the LIME model was incorporated to provide interpretations.

As shown on the right side of Table 7, the DNN model interpreted by the LIME model assigns a degree of support and contradiction to the top five important variables of the Case No. 69, and both the "Rollcall" and "SubmissionPct" variables contradict the outcome "Average" class with a measure of negative weightings. The Figure 3 also illustrates the predictive probability of the "Average" class for the same case is 0.614 with related top 5 variables. The comparison of the top 5 important variables between the DT global model and DNN local model interpreted

by LIME model provided synthesis insight from different perspectives and different practices for understanding the failure students. The other two global models interpreted by the LIME model also provided supplementary results of important variables in Table 8. Since all three global models applied different ML and AI algorithms, some important variables of different models were even contradictory to each other. For example, the "FirstExam" variable was ranked as top 2 with a positive weighting of 0.390 by the DNN(LIME) model, whereas, the same variable was ranked as Top 1 with a negative weighting of -0.247 by the DT-DNN(LIME) model.

Table 7. Top 5 important variables (DT global model and DNN/LIME local model)

Global Model: **DT**		Local Model: **DNN(LIME)**	
Variables	Weights	Variables	Weights
SubmissionPct	1.000	FirstExam	0.390
SecondExam	0.849	SecondExam	0.254
CourseComplet	0.656	Rollcall	-0.109
MaterialViewPct	0.612	SubmissionPct	-0.061
FirstExam	0.565	SubjectNum	0.044

Table 8. Top 5 important variables (DT/LIME and DNN/LIME local models)

Local Model: **DT(LIME)**		**DT-DNN(LIME)**	
Variables	Weights	Variables	Weights
FirstExam	0.184	FirstExam	-0.247
SecondExam	0.151	ClassNum	-0.046
MaterialViewedPct	-0.067	MaterialViewedPct	0.042
SubmissionPct	0.064	SubmissionPct	0.049
WebLinkViewedPct	-0.024	AcademicYear	-0.035

To increase the explainable ability of the hybrid model based on the synthesis of the global and the local models, the compensatory and non-compensatory approaches were implemented. As the DT model generated interpretable if-then decision rules, the multiple antecedent statements of rules were combined with the variable importance derived from DNN(LIME) model to provide additional interpretability. In contrast to the DT model, the DNN model enabled the local model to focus on a subset of selected input variables to create a nonlinear model for improving predictive accuracy.

The non-compensatory approach searched for the duplication of variables in the if-then decision rules of DT model and compared them to the outcome of the DNN(LIME) local model. The variable importance from the local models were neglected because the local models only accounted for partial interpretability of a subdivision to the global model, and their effects were surpassed by the global model. On the contrary, the compensatory approach intended to consider the importance of duplicated variables derived from both models. As the antecedent statement of a decision rule comprised the conjunction or disjunction of compound inequalities, the importance of an individual variable within the compound inequality was compensated by the importance of the same variable acquired from the DNN(LIME) model. Moreover, to increase the explainable ability of the hybrid model based on the synthesis of the three local models, the Dempster-Shafer Theory (DST), also known as the Theory of Evidence, might be involved to resolve the conflicting weightings of variables between models in our further study.

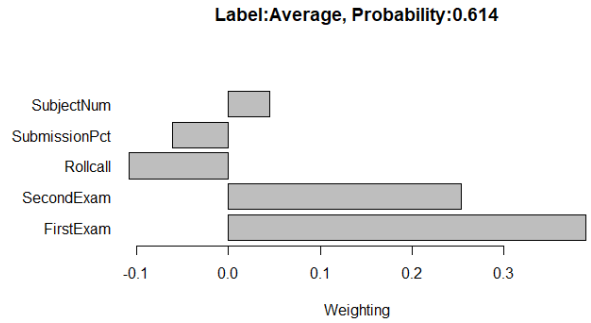

Figure 3. Prediction result of DNN/LIME model for "Average" class (Case No. 69)

3.3 Experiment Result for the Third Stage

The model fusion stage requires both the DT model and the KNN model to complement each other in the detection of the failure students, which were classified as "Bad" during prediction process. However, since all of the prediction models might encounter a problem with the Type I and II errors, which the actual "Bad" student been classified as "Average" and vice versa.

Table 9. Prediction result of all models (Case No. 69)

Models	Bad	Average	Good
KNN	0.151	0.849	0.000
DT	0.232	0.737	0.032
DNN	0.355	0.614	0.031
DT-DNN	0.256	0.743	0.001

Table 10. Prediction result of model fusion (Case No. 69)

Fusion Model (2): KNN Model					
Case No.	44	80	41	100	10
Rating	Bad	Average	Average	Bad	Average
P(Rating)	**Bad (0.569)** Average (0.431) Good (0.00)				

Fusion Model (1): DT Model	
P(Rating)	Bad (0.232) **Average (0.737)** Good (0.032)
Rule No. 2	FirstExam < 63.5 & SubmissionPct >= 0.635 & SecondExam < 51

All observations classified as false positives, false negatives and true positives in "Bad" class were recorded each time during the training of the DT model. These observed samples were appended to the training data set for training the KNN model later. The KNN model predicted the outcome of a new sample by comparing it to K similar cases in the updated training data set, while K could be specified by the user. The optimal K value was equal to 5 in this study, as the value was chosen based on minimizing the cross-validation error of the model. As shown in

Table 9, the student Case No. 69 was predicted as "Average" by each of the three global models and the grafting DT-DNN model with the probability between 0.614 and 0.849 in testing data set, which suggested us that student was most likely to demonstrate satisfactory performance. The DT model also provided an explainable rule No. 2 as evidence to support the inferential outcome and account for the reason why the student achieves satisfactory performance. However, as shown in Table 10, the experimental result of the stage three found the top 5 nearest neighbors, and both the top 1 and top 4 cases similar to case No. 69 were predicted as "Bad". The predictive probability of the "Bad" class was 0.569 that outperformed the other classes.

4. CONCLUSION

To evaluate the learning effectiveness of numerous students taking various courses within different faculties is an extremely labor-intensive task. However, sophisticated machine learning (ML) and artificial intelligence (AI) technologies provide solutions to understand the behavior of learners from their personalized learning profile. This study developed and implemented an integrated system comprising three stages to conduct teaching practice and continuous improvement of teaching quality based on collected LMS data. The first stage used DT, DNN and KNN models as global models, where each individual model was applied to predict students' learning effectiveness based on their online activities and academic results. Although the neural-network based DNN model achieved the highest accuracy of 81.4%, where 72% of the failing students were correctly identified as "Bad" class, the structure and parameters of the model are not easy for people to interpret and understand. Therefore, the second stage used a model grafting technique to combine a global model based on decision trees supplemented with a local DNN model, and the dedicated hybrid XAI model allowed users to select a desired tradeoff between the accuracy and interpretability for the model. The experiment results shown that the accuracy of the predictions made by the DT-DNN system increase from 74.3% to 78.5%, while 75.4% of the failing students were correctly identified as "Bad" class. In addition, all three models interpreted by the LIME algorithm generated a degree of support and contradiction, measured by positive and negative weights, for each variable.

In the third stage, another model fusion technique that integrates the DT model and the KNN model to complement each other to reduce Type I and II errors of model, where the actual "Bad" student been classified as "Average" and vice versa. The fusion approach merges the decision rules or variable importance with the outcome of for the hybrid model. The synergy of both models would help to identify the students at risk of failing their courses and reduce the type I and II errors of the predictive models for predictive performance. In order to monitor the learning effectiveness of students and reduce their failure rate, all three stages could be proceeded iteratively and implemented as an early warning system to continuously search for students at risk of failing a course prior to the midterm exam, final exam or at any time through the semester.

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Evaluation of Simulation Based Education Clinical Preparation Program for Unstable Patient Management

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ABSTRACT

In this study, the aim is to evaluate clinical transfer of unstable patient management competencies acquired by learners in simulated environment and evolution of their skills in clinical environment. Performances of 87 intern medical students of Acıbadem Mehmet Ali Aydinlar University that participated in two weeks long simulation based education program were evaluated both in simulated environment and in real clinical environment. Analyzes were performed using the MedCalc Statistical Software version 12.7.7, Ostend, Belgium Program.Observational performance scores of Group A were 18,68±1,70 over 24 point in simulated environment and 19,65±2,87 in first clinical test and 22,12±2,45 in second clinical test .Scores of Group C were 18,9±4,55 in simulated environment 16,36±4,2 in first clinical test , and 23,32±2,44 in second clinical test..Scores of Group D were 14,5±4,04 in simulated environment 19,32±3,76 in first clinical test and 21,87±2,78 in second clinical test.. Scores of Group B in simulated environment were 17,68±2,73 but Group B performances could not be evaluated in clinical environment due to pandemic.Simulation-based education is effective for improving the competencies of learners for management of unstable patients.

Keywords: Simulation based education, unstable patient management, clinical transition program.

INTRODUCTION

Critical patient management. is one of the most stressfull situations for the healthcare providers[1,2,3]. The area where students feel most unprepared is critical patient management[4,5].For this reason, courses, training modules have been applied and outcomes have been evaluated in many studies to provide these competencies[6,7,8]. Simulation based education (SBE) courses and programs have been found to be beneficial for preparing future physicians for emergency conditions[9,10,11,12.]

In our country, preclinical period of medical schools' educational methods mostly focus on computer-based or written documents. Therefore, the educational opportunity for solving complex real life problems is missing. In order to prepare students for critical patient management two weeks long SBE Clinical Preparation Program is developed.

The research purpose is to evaluate the effectiveness of the SBE clinical preparation program carried out in the simulation center of our university.

METHOD

Research Type and Research Design

In this prospective cohort study, the participants consisted of 87 interns studying at Acibadem Mehmet Ali Aydinlar University Faculty of Medicine in the 2019-2020 Academic Year. After getting approval of Institutional Ethical Committee and taking written consent from the participants all, the interns included without any exclusion criteria. The learners were divided into four groups in accordance with the internship groups. Group A consisted of 20, Group B 19, Group C 24 and Group D 24 participants. Intern doctors attended the SBE (Simulation based education)-Clinic Preparation Training Program, which lasted a total of 10 days before starting emergency medicine internship. The aim of this training program was to prepare interns for the clinic on potential life threatening conditions (PLTC) such as altered mental status, shortness of breath, chest pain, multi-trauma, cardiopulmonary resuscitation and acute abdominal pain. The program was carried out in Acibadem Mehmet Ali Aydinlar University CASE (Center of advanced simulation and education) Simulation Center, which includes real medical equipment and advanced patient simulators. Evaluation of learners' performances on management of critical patients in real clinical environment were carried out at Atakent Hospital of Acibadem University.

SBE Clinical Preparation Program and Emergency Medicine Internship Program

The program aims to enable learners to evaluate the patient in accordance with the algorithms of critical patient management and make the necessary first intervention. In accordance with the schedule of each day and in line with the session outcomes, after 40 minutes of theoretical training, a two-hour interventional skills session followed by 4-hour simulation scenarios and reflection sessions were held. Each scenario took 15-20 minutes to complete, followed by the reflection / debriefing session 35-40 minutes. At the end of the training program each student performance assessed by two assessors (faculty members experienced on SBE) unstable patient scenarios in the simulated environment.

Intern students' clinical performance analysis were done in university hospital. .Each student's performance is assessed by two assessors on the bed-site when a critical patient arrived to emergency department and the first assessment was done .Clinical performances are evaluated both during the first and last week of emergency medicine internship.

Research Questions

1-Could the learners reached defined competencies for critical patient management in the simulated environment?

The technical skills such as ABCDE (airway, breathing, circulation, disability, exposure) management are evaluated by using ABCDE approach checklist

2-Have the defined competencies in critical patient management transferred to the clinical setting and developed?

During the first week, the educators evaluated critical bedside performance of the learners in the emergency department.

During the last week of the Emergency Medicine Internship, learners' performances at the critical bedside with the ABCDE checklist were evaluated again.

Processing and Analysis of Data

The compliance of continuous variables to normal distribution was analyzed using the Shapiro Wilk test. Descriptive statistics (mean, standard deviation, minimum, median, maximum) were used to define continuous variables. The change between dependent and non-normally distributed continuous variables were examined using Friedman test, The change between two continuous variables suitable for dependent and normal distribution was examined with the t-test in paired samples, and the relationship between the dependent and two continuous variables not suitable for normal distribution was examined with the "Wilcoxon Signed Rank" test. The comparison of two independent and normally distributed continuous variables was made with Student's t test, and the

comparison of two variables that were independent and not compatible with normal distribution was performed with the Mann Whitney U test.

Statistical significance level was set at 0.05. Analyzes were performed using the MedCalc Statistical Software version 12.7.7 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2013) Program.

RESULTS

87 interns who started the internship training period at Acıbadem Mehmet Ali Aydınlar University in the 2019-2020 academic year participated in the study. Learners did not attended to any kind of unstable patient management training before this preparation program so none of them have prior experience on the management of critical patients.

Assessors of the simulated environment were two SBE experienced faculty member of university who are responsible for simulation based preparation program. Inter-rater correlation was found 0.79.Assessors of clinical performance were two experienced faculty emergency physicians who are responsible for both running the emergency department in university hospital and training intern students. Inter-rater correlation was found as 0.82.

The Simulation-Based Training Program Outcomes of Intern Students on Critical Patient Management

In the context of the first research problem, technical competencies for critical patient management were evaluated . On the 10^{th} day of the SBE program, the learning outcomes were evaluated using the unstable patient management checklist via simulated patient scenarios in simulated environment.

As seen in Table 1, the test scores out of a total of 24 points were 18.68 ± 1.70 in Group A, 17.68 ± 2.73 in Group B, 18.9 ± 4.55 in Group C, and 14.5 ± 4.04. in Group D. The scores over 14.4 (%60) is defined as successful.

These scores show that although the scores are higher in some groups, each group has achieved the targeted level with the SBE program. A statistically significant difference was found between the scores of the groups in the analyzes (Kruskal Wallis, p <0.001)

Table 1. Comparison of performance scores of the groups evaluated in simulated environment with the Kruskal Wallis test

	Performance scores				***Kruskal Wallis Test***		
	Mean	SS	Median	Min-Max	*Chi-Square*	*df*	***p***
Group A	18,68	1,70	19,0	16-23	18.125	3	**<0.001)**
Group B	17,68	2,73	18,0	12-23			
Group C	18,90	4,55	19,5	8-24			
Group D	14,50	4,04	14,5	7-22			

Transfer of Intern Students' Simulation-Based Training Program Achievements to the Clinical Environment and their Advanced Development

As seen in Table 2, the mean scores of the groups for the SBE Program in simulated environment were close to the average scores obtained in the first week of the Emergency Medicine Internship clinical environment(First Clinic Test)

In the last week of the Emergency Medicine Internship(Second Clinic Test), the scores were increased in all three groups. Due to the

pandemic, the clinical performance analysis could not be performed in group B.

In the analysis performed with the Kruskal Wallis test, a statistically significant difference was found between the groups in the First Clinic Test ($p < 0.05$). The differences between groups in the Second Clinic Test were not statistically significant ($p > 0.05$).

Table 2: Distribution of performance evaluation results by groups

	Groups and Performance Scores				
	Group A	**Group B**	**Group C**	**Group D**	
	Mean +SS Med (Min-Max)	Mean +SS Med (Min-Max	Mean +SS Med (Min-Max)	Mean +SS Med (Min-Max)	***Kruskal Wallis Test***
SBE Program	18,68 ±1,70 19,0 (16-23)	17,68 ±2,73 18,0 (12-23)	18,90 ±4,55 19,5 (8-26)	14,50 ±4,04 14,5 (7-22)	*Chi-square =18,125 df=3* ***p <0.001***
First Clinic Test	19,65 ±2,87 20,0 (14-24)	Pandemic	16,36 ±4,20 16,5 (6-22)	19,32 ±3,76 20,0 (10-24)	*Chi-square =7,873 df=2* ***p=0,020***
Second Clinic Test	22,12 ±2,45 23,0 (15-24)		23,32 ±2,44 23,5 (18-24)	21,87 ±2,78 22,0 (16-23)	*Chi-square =4,430 df=2* ***p=0,109***
Friedman test	*Chi-square =18,264 df=2* ***p= <0.001***		*Chi-square =27,90 df=2* ***P=0,001***	*Chi-square =24,99 df=2* ***p=0,001***	

DISCUSSION

In this study the effectiveness of the SBE clinical preparation program was evaluated by observing the performance of the intern students on critical patient management both in the simulated environment and then in the real clinical environment.As a result of the performance evaluation, the groups acquired the targeted competencies in simulated environment but the differences between the groups were found. When the transfer to the clinic and the development process after it were evaluated, it was determined that the transfer was provided in all groups and developed until the end of the internship without significant difference between groups.The opportunity of working together in a safe environment prior to clinic educators and students enabled to get to know each other better and to be aware of the performances individually .

Watmough et al[12]. applied unexpected patient scenarios to senior medical faculty students in their study and evaluated the program with questionnaires immediately after the end of the education and immediately after the learners became doctors. As a result of the training conducted in a simulated environment after graduation, the learners found that it got easier to cope with unstable patients in the real environment. In our study, we could observe the actual benefit and transfer of skills to real clinical environment by assessing learners' performances at the bedside in real clinical environment.

Simulation acts as a bridge between classroom lectures and clinical practices as a training method[13]. Simulation is still considered as a very good training strategy in terms of providing the participants with the opportunity to apply their theoretical knowledge with scenarios in a simulated environment without putting patients at risk, and conceptualizing the knowledge under the mentorship of the trainers with debriefing and observing how the existing cognitive schemes are reflected in the practice[14,15].Integration of SBE into curriculum is important for preparation of students to real clinical environments.

CONCLUSION

Simulation based education offers learners the opportunity to convincingly experience complex processes in a real like clinical settings without the risk of harming the patient.Real clinical experience is important for improving critical patient management skills.

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The (failed) effort for a systematic approach to legal education in Albania

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ABSTRACT

Citizens' knowledge of the law plays an essential role in any democratic society where human actions and relations are regulated by legal norms and rules. As these rules are often complex and subject to change, and more often than not, based on a generalized perspective rather than a personal one. To establish and maintain an effective justice system, it is necessary to guarantee public access to the knowledge of law. This article will focus on a new approach established in Albania that public knowledge of the statutes, rights, obligations, etc., is not only a legal education function, but also a component of the dominant culture and of the civic need for access to the justice system. The article will argue how this approach, although admirable and worthy of effort, needs careful consideration and realistic planning, in order not to remain just a beautiful wish on paper.

Keywords: legal education, public education, justice reform, systematic education.

1. INTRODUCTION

The Strategic Plan for the Judicial System, 2019 – 2020, opened with this quote *"from Goethe"*: *"Whatever you can do, or dream you can do, begin it. Boldness has genius, power, and magic in it!"* [1] The famous misattribute of this quote to Goethe is a perfect representation of what is happening with the legal education in Albania: a lot of enthusiasm, bold plans, but little ownership and real life results.

During the past four to five years, Albania has been undergoing a complete overhaul of the judicial system. The reform process started with an Analysis of the Justice System in Albania (2015) [2], upon which the Justice System Reform Strategy and its Action Plan (2015) [3] were based. The article will analyze these documents in details regarding their findings on the lack and the need for institutionalization of legal education for the public and how has this impacted the need for an overhaul of the justice system. The goals of the Strategy in the field of legal education for the public will be analyzed *vis á vis* the subsequent constitutional amendments [4], as well as the package of laws enabled the implementation of this key reform.

The article will then elaborate on how the above was translated into goals, centered around the need to enable citizens to find their way around the broad legal spectrum, to recognize their rights and obligations, and the requirement to enhance the role and place of legal professionals in this process.

The article will continue to elaborate on how these goals were transposed into a comprehensive National Strategy for Public Legal Education (2019). [5] This strategy, that covers the years 2019 – 2023, is based on four main strategic goals that were designed and later detailed in an action plan in thorough consultations with the network of professionals that worked on the justice reform.

An analysis of the strategy will look into the real need to implement the strategy's goals or the ability to implement the respective actions. Furthermore, the comparative analysis with the effects of the justice reform will look into the possible outcomes of such overhaul in the education system. In the end the article will argue whether the political rhetoric that has accompanied the long and painful justice reform process have any type of effect of the public knowledge of the law.

Finally, this article will attempt to address the deficiencies of a public education reform that, while looking great on paper, has failed to devise real and attainable goals and has failed to undertake actions that are realistic and effective towards the desired result.

2. PUBLIC LEGAL EDUCATION AS PART OF THE JUSTICE REFORM

At the start of the judicial reform process in Albania, it was established that public access to the knowledge of the law is fundamental to guaranteeing an effective justice system [2]. The drafters of the judicial reform considered that informing and providing knowledge about the law, on rights, institutions law enforcement, responsibilities, etc., are not only related to legal education, but are components of culture and civic need for access to the rule of law. [2]

Understandably, the legal knowledge of the public directly affects the relations between the public and state agencies. People that are aware of the legislation and of their legal obligations are able to understand state functions better. They are able to address the appropriate agency, to formulate their requests and complaints better. In addition, a better understanding of the law has direct effects in improving the relationships between people, whether in their family, work, civil relations, protection of health, personal safety, environment, etc. Presumably, the knowledge of law encourages people to obey the law, avoid illegal behavior and reduce conflicts. [2]

The Analysis of the Justice System in Albania conceived a reform based on seven basic pillars. One of them was "Legal education and public legal education." The Analysis bases the need for better public legal education on these findings:

1. State institutions do not have projects and plans to promote and enable this form of education.
2. There is no national strategy for the legal education of the public.

3. There is no good coordination and planning for the continuing legal education of the public.
4. There is no division between institutions and the civil society of the tasks of education and continuous legal information for the public.
5. There is no institutionalization of legal education for citizens. There is no responsible institution for promoting, organizing and monitoring this activity.
6. Public legal education has been carried out through civil society initiatives, funded by international donors.

These findings are not supported by relevant sources in the Analysis. They seem to be the reading of the situation by the experts that have drafted the analysis. The experts have analyzed the curricula of pre-university education and these documents seem to support a finding that legal information is provided from the lower grades throughout the mandatory education, in accordance with the knowledge and abilities of each grade. [6 through 12] The Analysis does not provide any source or study to show what the level of legal knowledge is in the general population and what are the real needs of the general population in this regard.

In line with the experts' findings, the Analysis identified the goals and objectives to be achieved in the field of public legal education as part of the justice reform:

1. Provide the necessary information and develop the necessary knowledge, increase the capacity of citizens to understand and the skills needed to make the right decisions in situations related to legal rights and obligations.
2. Enable citizens to find their way within the broad legal spectrum, by recognizing their rights and obligations, and train then to identify and avoid potential problems in the future.
3. More participation and role for legal professionals.
4. Support the citizen to recognize the rights, understand the role and functions of institutions and mechanisms for the administration of justice, understand the concept of citizenship in the rule of law, update their knowledge in areas of their interest in the legislation.

The Analysis suggests a number of activities to accomplish the goals, such as massive information and media campaigns, distribution of informative materials on specific legal issues, publication of success or failure stories to highlight right or wrong, more information on auxiliary services to promote access to justice, a legal information website, open days in various state institutions and meetings of the public with law professionals.

The Justice System Reform Strategy, that was adopted on the basis of the Analysis set raising the public awareness of the importance of law enforcement as the main objective for the legal education of the public. The strategy lists these specific actions for the implementation of this goal [3]:

1. Inclusion of legal education in pre-university curricula.
2. Training of teachers through legal education in university curricula.
3. Increase practical legal knowledge and promote public activism.
4. Establishment of legal information websites.
5. Information campaigns on conflict resolution alternatives to dissuade from self-judgment and revenge.
6. Information programs for people in contact with the justice system.
7. State funding for legal information books.

Although many of the actions listed in the strategy seem to be a repetition of the goals themselves, the strategy is accompanied by an Action Plan (AP) where specific activities are listed with the aim of implementing the above actions. [3]

3. THE NATIONAL STRATEGY FOR PUBLIC LEGAL EDUCATION

As evidenced by a survey conducted by the Annenberg Public Policy Center (APPC) of the University of Pennsylvania, only a quarter of Americans can name all three branches of government. Nearly a third of Americans cannot name any of the three branches of government. [13] This survey support the argument that a lack of basic civics knowledge is worrisome and an argument for an increased focus on civics education in the schools. [14]

Despite a lack of similar surveys and studies in Albania, the lack of legal information in the general public was recognized by many as an issue to be addressed even as part of the comprehensive justice reform.

The National Strategy for Public Legal Education (SELP) was adopted by the Albanian Parliament in 2019. The SELP was drafted during the heated phase of the implementation of the justice reform. Admittedly, the strategy tries to address not only public legal education, but also the dynamics of the justice reform.

The process for drafting SELP started immediately after the adoption of the justice reform strategy. To determine the content and policies of public legal education, the Special Parliamentary Commission for the Reform of the Justice System set up a High Level Expert Group. The group initially relied on the findings of the Analysis of the Justice System and on the main directions of the Strategy. The group, then, found it necessary to treat public legal education beyond its formal concept and to include, in addition to educational institutions, other actors, public and private, in order to expand and extend legal education to all the levels of the society. Data was collected from different institutions and actors that play a role in the field, and research work was conducted to identify the applicable international legal and policy framework.

SELP establishes the following strategic goals for years 2019 – 2023 [5]:

1. Improvement of the institutional organization and functioning in offering public legal education.
2. Guarantee and strengthen effective institutional cooperation at local, regional and national level for the realization of public legal education.

3. Ensure effective and efficient performance of public and independent institutions, the media and civil society.
4. Awareness of the public regarding the importance of knowing the law, their rights and obligations, based on specific needs and on the promotion of civic activism.

Each strategic goal is followed by the measures of their implementation. The measuring indicators are established in number of legislative actions, memorandums, number of training or informative sessions. SELP does not contain measuring indicators that assess the real knowledge of the public regarding the law and their legal rights and obligations that would support arguments for improving it. [5]

What seems to be a real shortcoming of the strategy is the argumentation of the way the provided action will contribute to as better understanding of the law by the general public. On the other hand, SELP does not evaluate the resources of the institutions it charges with actions. An effective strategy would need to aim toward reaching results in realistic terms, without excessive provisions and without overloading the structures that will be assigned to the implementation. [15]

4. THE IMPLEMENTATION OF THE STRATEGY

The justice reform in Albania was heavily supported by donor funds. Subsequently, there was a lot of funding dedicated to the monitoring of this reform too. Although many experts involved in monitoring activities were the same that drafted the reform, their reports help create un understanding of the impact of the reform on legal education and public legal education.

The monitoring reports were mainly based on desk research, official information from stakeholders and semi-structured interviews with responsible officials. The reports do not include surveys of public perception, information or knowledge.

In 2019, the only progress reported regarding the legal education of the public remains the work for drafting the SELP. [16] Regarding legal higher education, the reports mention that a number of concrete legal steps have been taken. These action, of an exclusive legislative nature, were aimed at improving the quality, as well as addressing the specifics of legal education. [16] Despite this, the newly enacted legislative measures have remained in paper and the obligation arising from them practically unenforceable due to the failure to issue the relevant regulation that would establish specific duties and deadlines. [16] similarly, the processes of evaluating the quality of higher education and the capacity building in legal research have started, but remain largely stalled and not completed. [16]

In 2020, the reports on the justice reform continue to list the approval of SELP as the only progress in the area of public legal education. [17] These reports echo the same concerns as above regarding higher legal education. [17]

5. THE BROADER EFFECTS OF THE JUSTICE REFORM

The justice reform should have been considered at its peak in 2016 when the Constitutional Amendments were approved. Instead, the Constitutional Amendments, more that the start of real change were a continuation of the political rhetoric that accompanied the justice reform. Until 2019, the justice reform stalled amid political accusations and counteraccusations. During these 3 years, the justice reform was practically reduced to the vetting process of judges and prosecutors and little success was achieved in other areas. [18]

The year 2019 marked the transfer from the rhetoric to the actualization of the justice reform. The time elapsed until then, considered by some as a period of transition between the old and the new system, [18] encountered obstacles that prevented the country for re-establishing a Supreme Court and a Constitutional Court after all their members failed the vetting process. It is evident that in this situation, the focus has been directed miles away from the concerns of legal education, much less those of public legal education.

Precisely, one of the criticisms addressed to the justice reform is that it left out legal education. The Justice System Analysis and Strategy dedicate specific chapters to legal education, but the implementation of the reform has not really addressed any of the issues identified by the Analysis. [19]

During the reform process, the discussions included amendments in Law No. 80/2015 “On higher education and scientific research in higher education institutions”. The aim was to amend the duration of legal education and require five-year integrated second cycle degrees. But, the most notable proposal was the inclusion of a “bar exam” – an examination at the end of law school that would give graduates the license to practice law in Albania. The draft law “On the state examination for lawyers” was, in fact, part of the justice reform legal proposals and was submitted twice in the Parliament (28.12.2016 and 22.09.2017), but after resistance from the academic world, was withdrawn both times. [19]

Legal education in Albania faces today many problems, some of them consistent with the concerns regarding higher education in general. Legal education suffers from lack of literature and academic research, little focus from professors, that while employed as academics, have widely known careers as defense lawyers, civil society activists and consultants. These factors, together with the large number of law graduates from public and private universities, have contributed to a diminished reputation and regard for the legal profession in general.

The public tends to view legal knowledge as a second-tier knowledge, something that we all possess, because it is evidently so easy to get a law degree and practice the legal profession. In addition, the appearance of obvious conflicts of interests have diminished the reputation and regard for law academics in Albania both in front of law student and in front of the public.

The public and political discourse on the justice reform have also had their negative impact on the way legal education was left outside of the real efforts undertaken as part of the reform. Reporting on justice reform remains a challenge for the Albanian media and journalists, especially because of the fundamental changes made to the entire justice structure. [20] Journalists have difficulties in understanding the functions of new institutions, which leads to truncated and inaccurate reporting. [20] For those reasons, the information that gets to the public is very often inaccurate and distorted.

In addition to this understandable difficulty, media in Albania suffers generally from financial shortages, lack of stability and

political influence. [20] To this, we must factor the heated political discourse on justice reform, which is one of the decisive factors in the way the Albanian public perceives the law and justice institutions.

The political majority continues to treat justice reform as its political promise. It identifies with the reform and it rejects all criticism. [21] The main opposition parties fail to distinguish between positive and critical developments in justice reform. They appear to be just against it, which undermines confidence in the long-term effects of the reform. [21]

The political subjects, journalists, publicists, activists involved in the discourse on judicial reform show a lack of in-depth knowledge of the basic concepts of justice reform, vetting and the essential elements of reform, by some of the subjects monitored in this study. This is also true in the media: a general lack of reference to the relevant legal framework. [21]

6. CONCLUSIONS

Justice reform seems to have ceased to be the main priority in the political agenda, although it has been the most important development in Albania for quite some time. [18] The political atmosphere surrounding the justice reform is very controversial, but, at the same time, the main political parties are *de facto* against an effective actualization of the aims of the reform. [18]

The justice reform affects not only the legislative process and the judiciary, but also every part of the public life in Albania. It especially affects the legal culture in the society by creating justice institutions that people can trust. The increased trust in institutions will significantly influence the overall development of society. It is therefore necessary to stop the militant and personal discourse on the justice reform and to accomplish its written goals one by one, from the Constitutional provisions to the publication of the book "Law in your life.".

Justice reform should be considered as a common national objective of the entire Albanian society and institutions. Only then, legal education will have a chance that goes beyond the expression of goals in a strategy.

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Covid-19 Context for Meta Assessment of Digital Learning On European and Oriental Languages Programs

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ABSTRACT[1]

The study objective is to estimate and critically review the context for meta-educational assessment of digital learning on European and Oriental languages programs via in-depth assessment of individual digital distance and hybrid learning experiences and best practices by students of European (English, French, Spanish, Italian) and Oriental (Mandarin Chinese, Japanese) Languages university level programs in different regions of Ukraine (Eastern Europe) through the span of educational activities in the time-frame of COVID-19 quarantine measures of March 2020 to January 2021. The meta-assessment survey and analysis of different ICT tools is used to estimate the comprehensive array of parameters and challenges of individual quality and efficiency of translation of the real life Foreign Languages Acquisition practices into digital blended format, involving activation of interdisciplinary skills and cross-sectorial activities, assisted by ICT tools. The designed meta-learning inquiry outcomes in this respect are: student satisfaction with the framework of digitalized foreign languages education; estimation of advantages, disadvantages, challenges and opportunities of e-learning and hybrid learning in the framework of COVID-19 lockdown; evaluation of e-learning and hybrid learning efficiency as compared to traditional, face-to-face learning formats for foreign languages programs; meta-assessment of digital learning process design, learning outcomes and acquired competencies for university programs of Oriental and European languages.

Keywords: meta-assessment; digital learning; digital education meta-model; European and Oriental Languages Programs

1. INTRODUCTION

The global pandemic and quarantine measures have imposed challenges on the complex structures and procedures of higher education workflow. These restrictions influenced the scope of individual experiences, educational and meta-educational outcomes, and estimated quality of higher education in countries across the world.

This research focus is the assessment of individual experiences and efficiency estimation of complex models of digital learning. The meta-educational significance of the study is informed by the inherent understanding of ***meta-learning*** as *"the process by which learners become aware of and increasingly in control of habits of perception, inquiry, learning, and growth that they have internalized"* [22].

The meta-educational dimension of presented study is therefore disclosed through such *avenues of inquiry*: 1) assessment of individual experiences and quality estimation of e-learning and hybrid learning in the framework of COVID-19 lockdown for university programs of Oriental (Mandarin Chinese, Japanese) and European (English, French, Spanish, Italian) languages; 2) assessment of individual experiences and quality of e-learning and hybrid learning in the framework of COVID-19 lockdown as compared to traditional, face-to-face learning formats; 3) individual quality assessment of learning process design, programmed leaning outcomes and projected competences for university programs of European and Oriental languages in the framework of COVID-19 lockdown.

The presented study is a parcel of comprehensive institutional inquiry into the toll digitalization and amplified use of ICT tools put on different aspects of Oriental (Mandarin Chinese, Japanese) as well as European (English, French, Spanish, Italian) languages acquisition efficiency, assessment management, programmed results, communicative and digital competency formation in COVID-19 lockdown paradigm.

The accumulated data so far has been seminal for a range of publications [19; 20], covering the issues of ICT tools efficiency rating regimen for foreign languages programs, isolated and comparative case studies of Oriental and European languages acquisition digitization procedures and best practices.

The estimations of digital distant education state-of the-art, structure and quality, conducted across various studies in the pre-COVID-19 inquiry framework have spanned, among others, assessment of satisfaction with distance learning experience [2; 3]; added value of online learning [27]; evaluation of learning

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outcomes [7; 23]; overall attitudes to distance learning [25]; challenges of online education [21]; networking principles in e-learning [26].

These issues require a comprehensive revisit in terms of the toll the global pandemic took on complex framework transformation of educational formats to digital remote mode, which informed the meta-learning experiences and subsequent shift in quality estimations of linguistic education in universities due to the abrupt transition to exclusively distant, digital distant or hybrid learning formats as a mode of formal education and university degree acquisition (as opposed to being a mostly auxiliary learning method or an informal learning format).

This **research objective**, henceforth, is to estimate and critically review the context for meta-educational assessment of digital learning on European and Oriental languages programs via in-depth assessment of individual digital distance and hybrid learning experiences and best practices by students of Oriental (Mandarin Chinese, Japanese) and European (English, French, Spanish, Italian) Languages university level programs in different regions of Ukraine (Eastern Europe) through the span of educational activities in the time-frame of COVID-19 quarantine measures of March 2020 to January 2021. The survey and analysis of different ICT tools is used to estimate and meta-educational added value of the comprehensive array of parameters and challenges of individual quality and efficiency of translation of the real life Foreign Languages Acquisition practices into digital blended format, involving activation of interdisciplinary skills and cross-sectorial activities, assisted by ICT tools.

The study premise is based on identification of complex digital distant learning formats (u(biquitous)-learning [8], m(obile)-learning, b(lended)-learning, hybrid learning) and modes for university educational programs and projected digital literacy requirements [10; 12; 32].

The online survey study of 17 universities in regional centers and the capital city of Ukraine provided for disclosure of findings that inform the meta-educational assessment of digital learning quality and further potential for design of digital learning models for foreign languages programs in the framework of COVID-19 perpetuated quarantine measures. A sizable sample of 445 University students of different tiers (Bachelor's level, Master's level, Graduate school level) in European and Oriental Languages programs in Ukraine were surveyed in the time-frame of COVID-19 quarantine measures of March 2020 to January 2021.

The designed meta-learning inquiry outcomes in this respect are projected to be: student satisfaction with the framework of digitalized foreign languages education; estimation of advantages, disadvantages, challenges and opportunities of e-learning and hybrid learning in the framework of COVID-19 lockdown; evaluation of e-learning and hybrid learning efficiency as compared to traditional, face-to-face learning formats for foreign languages programs; meta-assessment of digital learning process design, learning outcomes and acquired competencies for university programs of Oriental and European languages.

2. FINDINGS

Meta-modelling of Digital Education in the Framework of COVID-19

Theoretical problems of complex, holistic, multidimensional modelling of reality and its separate spheres (one of which is the sphere of the latest computer technologies development) are directed by the deterministic interaction of objects, signs of their reception and interpretation (in the field of individual and collective consciousness), embodiment, consolidation and retransmission of the results of interaction of these systems of features.

In this regard, the fundamental dimension of being (as synonymous with the latter "reality", "realm" and "world" can be used) [1; 5; 17] is defined as a heterogeneous hyperonymic concept that can summarize the multidimensional features of the world order:

- the world that really exists and is subject to direct perception;
- a world that is not subject to direct reception, but exists in reality;
- a world that is imaginary, unreal (for example the ideal, mythological images);
- reality, which exists objectively, regardless of human consciousness (nature, objective physical laws of the world order, space-time dimensions);
- the general way of life of humankind, society, culture, civilization.

Thus, the Computer Being dimension is understood as a fundamental ontological parameter that exists outside the scope of human mind and is involved in the perceptual field regardless of the will of the subject of cognition.

Conditions for the development of modern globalization civilization determine the expansion and refinement of the paradigm of views on the theoretical principles of determining the groundwork and characteristics of the consolidation of the world order, its perception in culture, collective social consciousness and social activity.

The intellectualization of modern global culture determines a qualitatively new approach to understanding the processes of parallel development of human activities and cognitive (intellectual) experiences. That is the origin and methodological premise of the concept of "noosphere". The noosphere is defined as the current stage of development of the biosphere, associated with the emergence of humanity in it [16; 13], and is interpreted as part of the planet and planet ambient with traces of human activity.

The integral real component of the Noosphere is identified as the Technosphere - a set of artificial objects (technologies) created by the humankind, and natural objects changed as a result of technological activity of humankind [15; 18]. In turn, Computer Being (computer reality, cyberspace) is a complex, multidimensional, interdisciplinary sphere of synthesis of reality, human experience and activity mediated by the latest digital and information technologies; technogenic reality, a component of the technosphere of existence.

In the educational sphere, according to our estimations, the result of fundamental Technosphere shift, induced by the COVID-19 pandemic development and enhanced by subsequent digitalization measures, was the need to take quick comprehensive action [19] in order to achieve such desirable results: a) To adapt the existent complex educational models to digital, remote and blended formats; b) To activate comprehensive awareness and meta-learning skillsets, otherwise latent or underutilized in the educational process.

The underlying understanding of educational systems as a meta-negentropy phenomenon, that is - a spatial and a temporal ordering of the educative process [6], provides for estimating digital or electronic learning (e-learning) to be a form of ubiquitous learning (u-learning) that involves learning in an environment with full access to digital devices and services at any given moment [30].

It is postulated, that digital learning is a parcel of the Technosphere shift in comprehensive human activity [20]. As such, digital learning as a complex object system, dominated by

concepts of electronic interaction and communication, acquires the following features:

- ubiquity (inclusiveness);
- ontocentricity;
- integrativity;
- isomorphism;
- normativity;
- communicative substantiality;
- information capacity;
- interactive framework meta status.

Through the combination of these features, digital learning is tangent to the complex systems of outlook and cognitive activity, such as:

- Model of the world / worldview (combined features are: *inclusivity, integrativity, isomorphism*);
- Linguistic picture of the world (combined features are: *communicative substantiality; interactive framework meta status*);
- Noosphere (combined features are*: ontocentricity, information capacity*) – Figure 1.

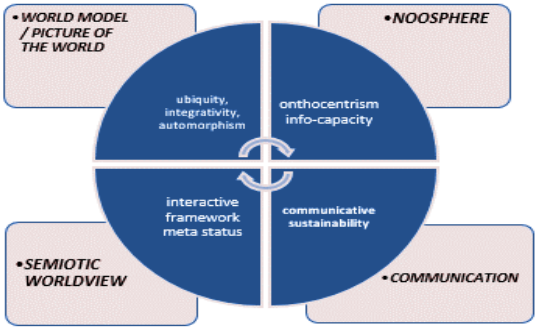

Figure 1: E-learning profiling against complex cognitive systems

The framework evolution and innovation of e-learning as a complex meta-system of human activity in the digital age, is determined by a range of qualifying conditions of its emergence, existence and transformation in the COVID-19 timeframe, including: 1) exhaustive synchronization of the object, phenomenological and anthropological planes of educational activities and proceedings with the processes of ICT development; 2) exhaustive output of isomorphism between ontological, anthropic (social, communicative) and digitized structures of reality and educational activity; 3) flexibility, adaptability and dynamic potential of the educational activities enhanced by ICT tools and digital technology (that is fulfilled, in particular through the information capacity, hybridization, evolution of the basic ontological and functional features of the learning process).

COVID-19 quarantine measures, put in place country-wide in educational institutions Ukraine in the time spans of March-June 2020, October-December 2020 and January 2021 have demanded the simultaneous employment and overlap of the following structurally complex learning formats:

- e-learning 1.0 (direct synchronous computer assisted distant instruction),
- e-learning 2.0 (mostly asynchronous computer-supported collaborative learning, also known as "networked collaborative learning") [28];
- blended learning (in-person teaching with asynchronous ICT assisted learning methods) [31; 9; 4],
- hybrid learning (synchronous instruction of in-presence and remote students via ICT tools) [11].

Modelling of the comprehensive meta-framework of educational activities and experiences in foreign language acquisition transformation into digitally enhanced format is, therefore, possible through an interoperable set of parameters (Figure 2):

- Time
- Space
- Communicative distance
- Dependence on ICT tools and infrastructure
- Level of complexity

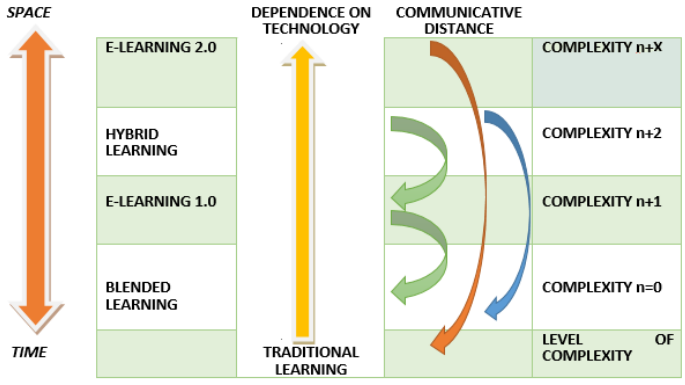

Figure 2: Meta-modelling of Digitally Enhanced Learning Formats

Based on the level of quarantine alert, adopted in regions of Ukraine, different level of complexity was utilized for educational activities in universities: e-learning 1.0/2.0 (for red and orange zones of quarantine) and hybrid learning (for yellow zones) formats were implemented.

Meta Assessment of Digital Learning On European and Oriental Languages Programs

Profiling and subsequent modelling of complex digitally enhanced learning formats that permeated the foreign languages acquisition landscape in the timeframe of COVID-19 measures informed the content and structure of the survey, conducted among Oriental and European languages primary stakeholders to assess the in-depth individual experiences among the estimated parameters of digitally enhanced distant learning models: 1) Complexity; 2) Subjective efficiency; 3) Information and communication efficiency; 4) Learning outcomes efficiency.

The designed meta-learning inquiry outcomes via in-depth assessment of individual digital distance and hybrid learning experiences and best practices by students of Oriental (Mandarin Chinese, Japanese) and European (English, French, Spanish, Italian) Languages university level programs in different regions of Ukraine yielded the following results across the board.

Student evaluation of the framework of digitalized foreign languages education.

Overall assessment of individual experiences and quality estimation of e-learning and hybrid learning in the framework of COVID-19 lockdown and quarantine measures (March 2020 – January 2021) for university programs of European (English, French, Spanish, Italian) and Oriental (Mandarin Chinese, Japanese) languages yielded the following representative results across the board.

The assessment of overall comprehensive individual experience (or a take-away) of e-learning and hybrid learning in the framework of COVID-19 lockdown by different groups of stakeholders was conducted according to the 5-Point Likert Scale (a response scale in which responders specify their level of engagement with a statement or a parameter in five points: (1) Strongly disagree; (2) Disagree; (3) Neither agree nor disagree; (4) Agree; (5) Strongly agree [14]. The extremum points for evaluation were 1 (maximum negative overall experience of e-learning and hybrid learning) and 5 (maximum positive overall experience of e-learning and hybrid learning).

Students of European and Oriental languages programs of all levels in universities of Ukraine evaluated their comprehensive individual experience of e-learning as predominantly 4 – mostly agreeable (34,9% of respondents), 3 – Average (30,7%), 5 – most agreeable – 21,05%.

The average estimation of the overall individual satisfaction with e-learning and hybrid learning experience on Oriental and European Languages programs by students of universities across Ukraine is 4>3 – mostly agreeable to average with an insignificant deviation range.

Qualitative meta-assessment of the e-learning and hybrid learning was conducted through the retrospective evaluation of respondents' individual experience through the span of March 2020 to January 2021, in order identify and select the preferred features and elements of e-learning and hybrid learning among the following:

- Ability to work from home
- Ability to customize of the study space (video conferencing or LMS)
- Adaptability and flexibility of the work schedule
- Save time for commute
- Parallel performance of several tasks and activities
- Opportunity to technologically diversify educational activity, educational materials and methods
- Ability to adapt the structure and content of curricula of disciplines to the conditions of online learning
- Ability to activate and improve different types of soft skills
- Opportunity to improve digital literacy
- Ability to work independently in the learning process
- No need for constant interpersonal communication
- Ability to engage international professionals through digital means

Quantitative assessment demonstrated that students of European and Oriental languages programs of all levels in universities of Ukraine have evaluated the meta-learning prominence of preferred features and elements of e-learning and hybrid learning and identified the significantly top ranking ones as follows: Ability to work from home (75,3% of respondents); Parallel performance of several tasks and activities (51,2%); Adaptability and flexibility of the work schedule (48,8%); Ability to work independently in the learning process (43,4%).

Estimation of advantages, disadvantages, challenges and opportunities of e-learning and hybrid learning in the framework of COVID-19 lockdown.

Top scoring features and elements of e-learning and hybrid learning, considered drawbacks, challenges or a hindrance by all groups of students of Oriental and European languages programs of all levels in universities of Ukraine are: Increased amount of workload in preparation for training sessions (63,9% of respondents); Emotional burnout (54,6%); Lack of interpersonal communication on a regular basis (47,4%); The need for specialized technical means of training and online communication / technical limitations (32%).

The following elements of hybrid and e-learning scored highest by students of European and Oriental languages programs in universities of Ukraine as *desirable to be retained and further implemented* in the educational process of any format outside of the COVID-19 limitation measures:

Electronic tests and assessments online -	70,3% of respondents
Use of e-textbooks and materials -	54,1% of respondents
Online classes through video conferencing -systems	52,5% of respondents
Communication through electronic resources - (mail, video, chat services, social networks)	50,8% of respondents
Ability to build an individual learning trajectory	43,6% of respondents
Ubiquitous learning -	40,4% of respondents

Meta-assessment of digital learning process design, learning outcomes and acquired competencies for university programs of Oriental and European languages.

The following elements of educational process were identified and selected by respondents across all groups and Foreign Language Programs, constituting a learning meta-design model:

- Conducting lectures
- Conducting practical classes
- Development of training materials and materials for assessment
- Execution of tasks (volumes, format, method of presentation)
- Organization of group work of students
- Organization of independent work of students

- Organization of creative work of students
- Organization of research work
- Communication with students (current)
- Communication with students (advisory)
- Informal communication outside the learning process / mentoring and moderation
- Assessment (formative, summative, qualification)

Each element of educational process design was subjected to ranking by all groups of students according to the 5-Point Likert Scale in terms of its efficiency for e-learning and hybrid learning quality assurance.

Students of Oriental and European languages programs of all levels in universities of Ukraine have identified the following elements of the educational process and management as ranking highest (*5 – potentially most conductive for quality of e-learning and hybrid learning in individual experience*):

- Conducting lectures (51,5% of respondents);
- Organization of group work of students (40,4%)
- Summative assessment (37,7%)
- Organization of independent work of students (27,1%)
- Communication with students (current) (24,3%).

The *standard deviation* of the top scoring e-learning design elements range is 9,7.

Subsequently, students of Oriental and European languages programs estimated the following elements of the education design and management as ranking highest (5) as *empirically most efficiently adapted for e-learning and hybrid learning format:*

- Conducting lectures (52% of respondents)
- Organization of independent work of students (41,4% of respondents)
- Assessment – all forms (34% of respondents)
- Conducting practical classes (33% of respondents)
- Development of training materials and materials for assessment (32,6% of respondents)

The *standard deviation* of the top scoring e-learning design elements range is 8,3.

Meta-assessment of mandatory program competencies formation in the process of online and distance learning by students of Oriental and European languages programs has ranked the following in linguistic training and employable soft skills:

- Digital literacy – 48% (of respondents)
- Self-development – 42,6%
- Self-organization – 39,5%
- Proficiency in written foreign language – 36,6%
- Time-management - 32,8%
- Critical thinking in a professional context – 30,3%

The *standard deviation* of the top scoring e-learning design elements range is 6,5.

3. CONCLUSIONS

The global pandemic and subsequent quarantine measures and restrictions have significant influence on the complex structure and procedures of higher education workflow, which informed the scope of meta-learning experience accumulation, projected outcomes and estimated quality of higher education in the realm of Foreign languages acquisition in all regions of Ukraine.

The survey results on the individual experiences and quality assessment of e-learning and hybrid learning in the framework of COVID-19 lockdown yield comprehensive data on the meta-educational parameters and challenges of e-learning and hybrid learning complex transformation of Oriental and European languages programs, common for students of higher educational institutions across Ukraine.

Overall individual experiences and quality estimation of e-learning and hybrid learning in the framework of COVID-19 lockdown and quarantine measures (March 2020 – January 2021) for university programs of European (English, French, Spanish, Italian) and Oriental (Mandarin Chinese, Japanese) languages are assessed as mostly agreeable to most agreeable by all groups of students across educational levels.

The qualitative assessment of the complex e-learning and hybrid learning framework was conducted through the retrospective evaluation of respondents' individual experience that helped identify the comparative coordinates of positive and negative dimensions of the latter. The invariant positive quality indicators for e-learning and hybrid learning across all groups of students surveyed are the opportunity for multitasking, digital skills improvement and lack of commute expenditure. The invariant negative quality indicators for e-learning and hybrid learning across all groups of students surveyed across Ukraine are the lack of interpersonal communication, technical impediments and psychological states (fatigue, burnout, stress).

Assessment of learning process design, programmed leaning outcomes and projected competences for university programs of Oriental (Mandarin Chinese, Japanese) and European (English, French, Spanish, Italian) languages in the framework of COVID-19 lockdown allowed to identify the types of challenges that impaired effective and qualitative adaptation of learning process design into hybrid and digital distant format as predominantly socio-psychological for students of all groups surveyed in regions of Ukraine.

The presented study is limited in scope to the indicative survey results, exemplifying the outlined meta-educational dimensions of e-learning and hybrid learning implementation for Oriental and European languages programs in universities of Ukraine, induced by COVID-19 measures. The perspectives of the inquiry include fine-tuned estimation of the in-depth subjective quality assessment and subsequent efficiency assessment of hybrid and e-learning in different regions of Ukraine; contrastive case studies of Oriental and European languages programs emergency digital distance format adaptations in the COVID-19 timespan in universities of regional and national status; contrastive case studies and best practices of Oriental and European languages programs transformation to hybrid and e-learning format in countries of Europe and Asia upon alleviation of COVID-19 restrictive measures.

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On the Design and Conduction of Virtual Workshops: Experiences from Going Digital

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ABSTRACT

Workshops (e.g., Innovation and Ideation workshops, Design Sprints, etc.) and virtual workshops play a major role in today's businesses. On the one hand, they provide a methodology for interdisciplinary collaboration, and on the other hand, they help teams solve problems by developing novel ideas, often for potentially new business opportunities. At the same time, workshop participants learn to apply various creativity techniques to solve complex problems holistically. These lessons learned support participants beyond a particular workshop. By being creative and working cooperatively, they experience a new way of working together that inspires future collaboration opportunities. In addition, workshops help to spread the thinking process behind creative and collaborative work and functions as an educational tool for corporate staff. Challenged by having had to transform our highly interactive workshops into the digital space, we have identified several practices to address these problems. We present our findings on overcoming obstacles of virtual online collaboration and discuss them from a meta-educational perspective.

Keywords: Virtual Workshop, Innovation Workshop, Virtual Collaboration.

1. INTRODUCTION

It takes more than a single idea to change the world. Still – or maybe because of that – innovation is what today's business world and its players, e.g., entrepreneurs and scientists working in applied research, are striving for. Finding novel and appropriate solutions for complex problems seems to be merely a game of chance. But with the right methods and techniques, hard work, and skilled people, innovation can be achieved. It is an act of creativity that requires thorough planning and execution. Achieving innovation and solving complex problems can be guided by the application of certain creativity techniques that vary in their exact shape and methods by the actual field they are applied to. Learning to use appropriate techniques is essential. "Eureka moments", where one is suddenly struck by the perfect idea and the correct plan for execution, are seldom. One can even argue that such "eureka moments" are simply the result of implicitly or explicitly following creativity techniques.

Innovation Workshops (IWs) are a great method for creating an innovation-friendly environment and setting the stage for creativity. Put simply, any workshop is about invited participants meeting at a set place and time to work towards a common goal. The format of each workshop depends on the goal being pursued. Workshops can be used in various contexts and endless variations of methods and techniques exist. Educating people to "be creative" typically means educating people in applying a certain toolset and mindset to enable them to learn on the job. A prominent example of how a large number of people are being educated while doing their job are the efforts of the software company SAP in their "design thinking" strategy [1]. In 2003, the company's founder Hasso Plattner invested in the Stanford d.school and injected the core concepts and thought processes underlying design thinking into the software company [2]. In 2007, he founded the HPI School of Design Thinking Potsdam to further support the design thinking education of the software company's employees.

Design thinking experts educated SAP employees in applying design thinking workshops. This was largely done in workshop settings where real problems were solved. This laid the foundation for the employees to organize and execute their own design thinking workshops in their teams and departments, slowly disseminating the design thinking know-how within the organization. In the meantime, it has become a cornerstone of SAP's way of working, even spreading to their efforts with university students through the SAP University Alliances program.

Design thinking is more than just workshops, but workshops serve as a way of solving business problems, while at the same time educating participants in creative problem-solving. In our business practice, we also plan and execute IWs for our customers, which in turn enables our customers' teams to solve the complex business problems they face. Reflecting on how we plan and execute these IWs also serves as know-how transfer for our customers. This can include the elicitation of requirements (requirements workshop), the generation of ideas (ideation or IWs), the joint modeling of (eco-)systems (Tangible Ecosystem Design) or the development of one or more prototypes (Design Sprint or Lean methods), to name just a few examples. These workshops can take place either as a face-to-face event at a physical location (offline) or in a virtual space (digital). Some workshops are facilitated more openly than others; here, the facilitators allow varying degrees of freedom for spontaneous detours. Nevertheless, all workshops, whether strictly or loosely designed, should be carefully conceived, planned, prepared, and conducted. After all, many participants come together and spend many hours of their valuable time in the workshop setting. Facilitators must therefore ensure that in the end a satisfactory result for all is developed, or that enough understanding has been established or ideas have been generated to be able to continue working.

Virtual workshops offer the same broad potential for problem-solving, finding innovation, and discovering new business

opportunities as offline workshops, but also entail specific challenges. The key difference to an offline workshop is that all participants in a digital workshop collaborate and communicate virtually. They use video- and audio-conferencing tools as well as online collaboration means. Apart from this fact, the intention is, of course, to be able to work in digital workshops in the same way as in on-site workshops. Planning and delivering a workshop is a challenge in itself. Digital workshops bring with them a whole series of additional hurdles. These are primarily due to digital communication, which will be described in more detail below, but also to the special requirements of interactive workshops in terms of preparation and moderation.

2. CHALLENGES OF DIGITAL WORKSHOPS

"Online is just not the same as offline." You may have heard similar phrases or thought this yourself [3]. People cut each other off more often or feel greater inhibitions about getting involved. The sense of connection may also be diminished. Although digital communication brings many advantages and enables location-independent, distributed work, we have experienced the specific disadvantages and their impact on workshop formats throughout several of our IWs, especially during the year 2020, when the global Covid-19 pandemic greatly changed the way we work.

Digital communication is less rich in physical stimuli than on-site collaboration. Many sensory impressions are lost that humans can normally rely on. Communication occurs on many levels, not just purely on the basis of content. Our body language says a lot about our social connection to others; eye contact is perceived differently when people are sitting in the same room than when they are looking into a camera, especially since "looking into the eyes" is not possible, and even the spatial distance between speakers influences our communication. A digital meeting greatly reduces our sensory input, as many subconscious cues are attenuated or absent altogether [4]. On the other hand, false stimuli can be added by a poor Internet connection or disturbing noises. This makes it more difficult to interpret content correctly. People are more likely to unintentionally interrupt each other, and expressions such as irony are more difficult to recognize.

"Zoom Fatigue" is a phenomenon that describes the increased effort involved in digital communication [5]. Digital communication is significantly more strenuous than physical interactions. The human brain subconsciously tries to compensate for the perceived "errors" (e.g., poor image or sound quality), which leads to increased cognitive load. Therefore, we perceive long video conferences as more exhausting than "normal meetings". Our brain runs at full speed to compensate for the lack of communication content [3]. As humans, we have many senses and process a wide variety of sensory impressions at the same time. Digital communication creates an overload of visual and auditory impressions while depriving all other senses, which, even if present, are not interwoven with the current situation.

Workshops thrive on social interaction. As described above, in an online setting this is only possible in a weakened form. Participants easily feel "disconnected" or are distracted by other channels such as e-mails, chat messages, or family members in the background. In the worst case, this causes them to disconnect and no longer interact with the group, or at least prevents them from responding adequately [4]. This effect is intensified by the different technical abilities of the workshop participants [6]. Those who are used to spending a lot of time in digital conferences have learned to interact socially online. They find it easier to build up relationships virtually than participants with less digital experience [7].

Paper and whiteboards are among the simplest and most flexible means of collaboration in physical meetings and workshops. Unfortunately, online tools can only replicate this flexibility to a limited extent. A quick doodle on a piece of paper, a moderation card, or a whiteboard is made and understood intuitively offline. Digital tools need to be learned and thus present an increased obstacle with regard to their usage. As a consequence, ideas can be lost if the insight was not jotted down due to a lack of tool literacy or fear of making mistakes. Even the creation of a simple drawing can become a challenge, and recognized connections and thoughts can be lost if they go undocumented.

3. LESSONS LEARNED FROM A YEAR OF VIRTUAL WORKSHOPS

In previous years, before the global Covid-19 pandemic forced us to adapt and conduct virtual workshops, we insisted on executing them on site, due to the aforementioned challenges. As a result of the transition to virtual workshops the last year, we were able to observe how these challenges affect our workshop's performance and come up with initial solution proposals.

Achieving a flow state is important

Many of the challenges of virtual collaborations can only be mitigated, but not completely removed, at least to the best of our knowledge. This can be further examined with the first of our lessons learned. We attended many virtual meetings and workshops, contrasting them with regular on-site events. By design, our workshops are demanding, for some even exhausting. The facilitators' role is to push the participants through the workshop, to energize them, and to make them achieve more than they had thought possible. To achieve this, we rely a lot on the facilitators' ability to serve as charismatic mentors guiding their mentees by providing the right amount of challenge and supporting them in achieving a flow state [8]. The challenge lies within the workshop's sessions themselves. Support is provided in the form of pre-created templates and guidance materials, helpful comments, and ad-hoc adaptations to the technique used in the session. All of this requires the facilitators to examine the atmosphere and mood of the workshop participants, listen to group discussions without disturbing them, throw in ad-hoc information and clarifications, and quickly change the rules of the current session with minimal effort and distraction for the workshop participants.

Virtual collaboration needs digital literacy

In a virtual workshop, a facilitator's ability to control the flow is limited. Due to the weakened social interaction and less stimuli-rich communication, many small cues can be overlooked. Following discussions without interrupting them is harder than standing across the room, just within hearing distance. The facilitator needs to enter the same virtual conference room (breakout room) and be visible to all other participants, making them aware of the facilitator's presence. Interrupting a discussion in a virtual workshop with small hints or guidance is less unobtrusive as in an offline workshop. The facilitator needs to interrupt the whole group, waiting for everyone to be silent in a virtual conference room, whereas in an offline workshop the facilitator can also just talk to one person of the group to later on add the facilitator's information in the discussion. Of course, one can send hints through the text chat functionality offered by many video conference systems, or post a note on the digital

whiteboard, but we have experienced that this means only slightly less interruption than with spoken hints. We found that digitally literate participants could use these hints with more ease, but when the workshop participants were less skilled in working with digital collaboration tools, they were easily interrupted. We also found that offering a multi-stage onboarding experience prior to the workshop had a positive effect on the participants' digital tool literacy.

1) We have created a description of the workshop rules in advance, a so-called "netiquette", which we distribute before the workshop. In offline workshops, many things are obvious, but in the digital domain, they need to be clarified, e.g., how to communicate when someone leaves their physical room to visit the restroom. Key information should be summarized briefly and succinctly so everyone will read it. For example, one can suggest that participants should have enough snacks and drinks ready so they do not have to go to the kitchen unnecessarily, and ask them to keep their e-mail program closed during the workshop.

2) At least one week prior to the virtual workshop, we give the participants access to the digital collaboration tools used in the workshop (i.e., Microsoft Teams for video conferencing and Miro for whiteboard collaboration) and prepare a small training with several tasks (e.g., "Add a sticky note to the whiteboard and write your name on it"). Participants can take the time they need to learn the most important functionalities of the tools, and also experience any technical issues early enough to resolve them. In such cases, we provide assistance in solving these problems (e.g., camera not being detected, being unable to access the whiteboard with a specific browser).

3) The third step in our onboarding process is to start the digital workshop at least thirty minutes early and ask participants who regularly have problems joining virtual meetings to use this time to test-drive their setup and solve any problems. This onboarding process enables all workshop participants to contribute from the beginning. Nevertheless, one should try to minimize the number of virtual collaboration tools used during the workshop to enable a quick learning effect.

Use high-quality video and audio equipment

Another important aspect is the usage of high-quality audio and video equipment. As a workshop facilitator, one should try to lead by example and invest in a strong technical setup. One can make a professional impression by using a high-quality camera. This could be a webcam, a digital reflex camera with external power supply, or a dedicated video camera. Additionally, the lighting setup improves the image and makes the person in front of the camera stand out clearer. Indirect lighting from the front is ideal, while side light or backlighting can be disturbing. Different color temperatures can also be distracting. Experts arrange three-point lighting, where two light sources come from the side (guide light and fill light) and another one from above, which "models" the head. Nowadays, however, a ring light is also often used, which illuminates the speaker from the front. This is already available for 15 to 50 euros depending on size and design. The background should not be too turbulent and not distract from the speaker. As long as the Internet connection allows, each participant should turn on the camera so that the necessary connectivity is established and participants are more likely to get to know each other and work together. It greatly helps the facilitators to get a better understanding of the group and enables them to address potential problems much earlier. As the facilitator, one should be careful not to look "down" on the participants. If the camera angle is too low, one can build an elevation out of books, for example, so that the camera is at eye level.

In addition to the camera image, good sound is essential for understanding each other properly and enjoying the event. Participants should check in advance whether their microphone is picking up their voice clearly. Head-mounted devices that combine microphones and headsets provide easy access to less error-prone audio communication, as these devices eliminate feedback loops by design and the close proximity of the microphone to the speaker's mouth allows the device to pick up the voice clearly without too much interfering background noise. Audio quality can be further improved by using dedicated microphones such as those used by podcasters or people producing videos for social media (e.g., YouTube). One can easily find many tutorials and reviews on these platforms.

If possible, the workplace itself can be further enhanced for good audio quality. The room used for recording should neither be too dull nor reverberant. Large flat surfaces such as walls without furniture can create resonance waves that make it hard to understand the spoken word. If participants are in the same room, it is advisable to use a shared microphone, even if everyone is participating via their own computer. If two or more participants use the same microphone and a common sound output, there will quickly be unpleasant noise from feedback loops.

Energy Level

Keeping the participants' energy level high is important. Many people are not used to looking at a screen for long periods of time, nor do they enjoy doing so. It is also very uncomfortable for people who are not used to sitting at a desk for long periods of time to stay in this position for hours. In offline workshops, we compensate for this by working with Play-Doh, doing small exercise sessions, having an engaging yet low-stimulus environment [9], and offering tasty snacks and drinks. In a digital workshop, one can only control or influence a few of these aspects and must therefore find ways of circumventing them. We advise workshop participants to prepare snacks and drinks and keep them in close proximity. As a special gimmick, we occasionally send a physical care package to the participants before a digital workshop. It includes sweets such as gummy bears, candies, or, depending on the weather, chocolate bars (unsuitable in the summer months). Tea bags or small drinks (non-carbonated) are also a nice idea for physical well-being. A labeled map is a good way to introduce the process and clarify the goals of the workshop. A card can be designed so that participants can hang it on their doors to indicate that they are not available to their spouses or roommates. Emoji cards can be helpful tools for indicating problems or agreement directly to the facilitator or co-participants. Especially the cards saying "Super" and "Get to the point 😉" have proven popular during our workshops. But eating gummy bears together also provided a point of reference and created joy. Participants shared a common experience, which enabled informal conversations.

One should keep in mind that breaks should actually be used as such, instead of being filled with other activities. Digital workshops are more intense than on-site workshops, due to their specific challenges. This is why we usually keep them shorter in terms of the number of hours per day, allowing the participants to respond to urgent issues after or before the workshop. To ensure that break times are respected, we set an alarm clock that is visible to all participants and start promptly after the break.

Participants will then take the times given seriously, because no one wants to be late and miss important things. Before the break, it is worth pointing out that people should give themselves screen-free time. For example, a short stretching video could be posted in the chat to prompt interested participants to take part in it, or taking a short walk could be suggested. We have received positive feedback from participants who followed these suggestions.

To get an idea of the participants' energy level, one can visually display their "fill level". This is a fun exercise and guarantees that all participants are considered. To do this, each participant holds their arm up in the screen area as highly as the participant is filled with energy, from 0% at the bottom to 100% at the top of the screen. This helps to determine whether a break is needed. Regularly providing something unexpected helps to keep the participants active. When team results are presented, we do not always have the same person present the results, or if we have a roundtable discussion, we do not always choose the same order, but add some flexibility. Additionally, unexpected guest lecturers or game units can help to make sure that the participants' energy level as well as their moral remains high.

4. A META-EDUCATIONAL VIEW ON VIRTUAL WORKSHOPS AND THEIR CHALLENGES

We have shown challenges and potential solutions for virtual workshops that we have discovered and used. They do not only apply to IWs used in business environments, but also to other interactive online sessions with a similar setting. Schools and universities face similar challenges, maybe less in frontal teaching settings, but in interactive formats. From our experience in teaching students in smaller university courses (approx. 20 regular participants), we conclude that guidelines like our netiquette can be helpful for students to feel more secure in the less familiar environment of virtual teaching. Just as we cannot expect students to automatically and seamlessly switch to being educated online, we cannot expect teachers or workshop facilitators to work with digital collaboration tools without friction. The solution guidelines we have identified are based on our knowledge, practical know-how, and intensive research about workshops in general and virtual collaboration tools in particular. We as employees of a research institute for innovations, software and systems engineering consider ourselves to be digitally savvy, due to the nature of our education and business practice. We acknowledge that researchers, teachers, lecturers, and business practitioners from other fields face larger obstacles in switching to an online setting.

We have discussed the educational perspective of workshops already and have shown examples of how they can be used to teach participants not only the techniques, but also the thought processes underlying creative processes. In the same manner, virtual workshops not only lead to creative output and a learning effect in terms of creativity, but also serve as examples of how virtual collaboration can succeed. This creates another challenge for researchers, teachers, lecturers, and business practitioners who plan, prepare, and conduct virtual workshops. They need to consider their actions from a meta-educational perspective on virtual collaboration as well. People participating in online courses and virtual workshops will learn what is useful and what is not. These lessons learned should be discussed and analyzed on top of the actual content. We should take the time and incorporate these learning processes into our business and teaching practice in order to enable iterative meta-learning on virtual collaboration.

Further research on the challenges of virtual collaboration and potential solutions for overcoming them is required. This involves different fields of research (e.g., psychology, medicine, information systems, and education) and should be approached in a multi-disciplinary way. For example, we have started discussing the effect of the background shown in one's camera image when participating in video collaboration. In our experience, the background varies a lot. Some people have dedicated workplaces for online collaboration, with clean backgrounds, while others need to work from their kitchen, living room, or bedroom. Do personal items visible in the camera image distract others or do they help to form a rapport and to socialize, by offering hints on the personality of the participants and the facilitator? Should one use virtual background images or blurring effects as some tools offer or do they hinder personal connectivity between video conference participants due to their fake nature?

5. CONCLUSION

We have shown and discussed challenges for virtual collaboration, especially for virtual workshops, and presented lessons learned by conducting workshops virtually that we had previously held offline only. One key challenge is the organizational and cognitive overhead caused by virtual collaboration, which not only affects workshops but virtual interactive classroom settings as well. Facilitators, teachers, participants, and students need to take time to understand and learn how to use new tools, such as video conferencing and digital whiteboards. On top, the hardware (audio and video equipment, workplace) used for participating in these virtual collaboration settings is something that should be considered, adding another layer of complexity. Off-line collaboration does not require these additional overheads and runs naturally.

The challenges and lessons learned we have identified and presented in this paper are a starting point for the design of virtual workshops and the examination of research on the topic area. We believe the consideration of the meta-educational perspective of virtual collaboration that is present when transforming to the "new normal" is of high relevance. With this paper, we want to encourage multi-disciplinary research on this topic. We could only find little related work in our research and hope that the global research community will understand the global Covid-19 pandemic as an opportunity. One can easily identify positive effects as well, such as a reduction of travel activities, e.g., for conference visits, which reduces the emission of greenhouse gases [10]. Also, people can be involved that were prevented from participation in the past due to mobility impairments or other disabilities. In a more narrow perspective, we would like to build on the work shown in [11]. We have discussed a research agenda for improving the documentation of Innovation Workshops. Virtual workshops offer new opportunities for improving the feasibility of means of documentation, as software tools are already used for collaboration and might support the documentation as well.

ACKNOWLEDGMENTS

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Indicators of schooling development

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ABSTRACT[1]

In Social Science, the indicators are often built empirically. That is, the quality and quantity of the data determines, in this field also, the construction of indicators and their robustness. Because of insufficient information, one often proceeds by approximation to find the parameters of the theoretical distribution law. This also holds for education. The results obtained in this way should be readjusted when one has more information. One cannot, for example, continue to use the gross rate of admission or schooling when the age distribution of pupils exists. It is now unacceptable to find in some scientific publications a rate of schooling over hundred percent when we are sure that there are children out of school!

Such indicators, even if they are useful for an international comparability, are mathematically not very robust and may not correspond to local reality. That is, they would not correspond to the national needs for planning.

In the following, we propose another estimator to give the number of children registered at school by generation (children having the same age). Considering the quality of the educational data existing now in a great number of countries, one can achieve this goal with more statistical robustness. We have just to find the statistical law, which would generate these data in order to find their parameters. Once these are found, it will be easy to represent more accurately the reality studied and to better plan for educational policy.

Keywords: Statistics, estimator, education, rate, access

1. INTRODUCTION

With the UN Sustainable Development Goal 4 (SDG4): "Ensure inclusive and equitable quality education and promote lifelong learning opportunities for all by 2030", after the UNESCO goal: "Education for All (EFA) by 2015", it become necessary, for the countries that are not yet reach the universal schooling, which is the case of most African ones, to conduct a continuous assessment for determining the level of educational development. That is to know the scale of the effort to be furnished for that purpose. In other words, one tries in these countries to know how the schooling level evolves by generation.

The Admission's Gross Rate (AGR) and the Admission's Net Rate (ANR) are, until now, used as the indicators which give an idea about the proportion of the children in school per generation. But, these two indicators do not always reflect the reality. So they can lead policymakers or planners to mistake.

The Admission's Gross Rate, which is a ratio, not a proportion in percentage, between two incomparable populations on the basis of age, can be biased from the statistical point of view. Representing the number of all children who are in the first year at elementary school, its use will overestimate the proportion of children admitted at school. Its value can be hundred percent even if the total target population is not yet at school [1].

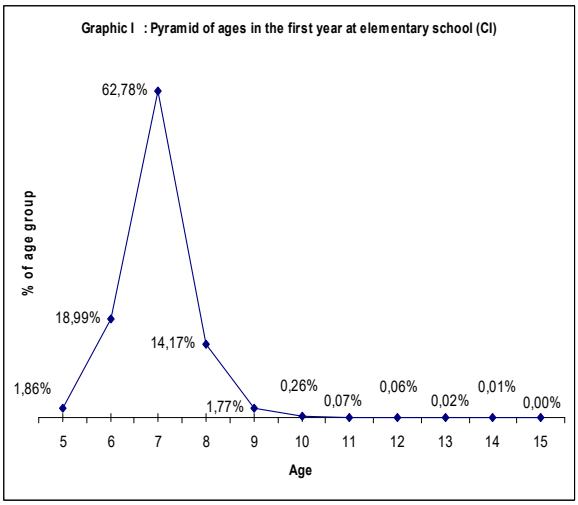
The Admission's Net Rate, if the legislation about the minimum age for admission to primary school was respected, would be a better estimation of the schoolchildren proportion per generation. But, this is not the case in many countries concerned by UN goal. The group of children having the legal age to be in school can be scattered into different levels. One can find them in the first, second, third or fourth year of the primary school (See Graphic I). Hence, when one limits oneself only to the pupils enrolled in the first school class with the required age, he underestimates the genuine number. Knowing that the value of this indicator will never reach hundred percent, even if all the target population is at school.

These two indicators, when the information can't be improved,

[1] I would like to thank Malick Thomas, Almamy Konté, Assane Diakahté and two unnamed readers of the manuscript for their helpful criticism.

might be used to have an idea of the reality. They could also be used for international comparability [2]. But, one must be aware that they are not statistically robust. That is mean, if there are more complete data about the studied reality, one can find better indicators.

Hereafter, we propose a less biased estimator of the number of children enrolled per generation, say the Generational



Admission Rate (GAR). Considering the quality of the school data existing at the moment in many countries, even the African ones, and the computer software development, it should not be difficult to calculate this indicator.

2. INDICATORS

To build an educational access indicator, one must take into account four parameters: the *level* in which students are enrolled, the *school year*, the different *ages of students* and also the *entry date* into School, which gives formally :

$$\boldsymbol{P_{t,\,k}} \qquad (1)$$

the total population of $\boldsymbol{k}$ years old at time $\boldsymbol{t}$

$$\boldsymbol{P_{t,\,k,\,d}} \qquad (2)$$

the number of schoolchildren among them at date $\boldsymbol{d}$. Hence, the admission rate will be, by definition :

$$AR = \frac{\sum_{d}^{D} p_{t_o,k_o,d}}{p_{t_o,k_o}} \qquad (3)$$

corresponding to the proportion of children of $\boldsymbol{k_o}$ years old, during the school year $\boldsymbol{t_0}$, found in the school at various date $\boldsymbol{d}$. This includes all members of the generation getting into school at the normal age $\boldsymbol{k_o}$, before this age or after. It is clear here that, simple observations don't permit to get the accurate number. To have this, it will be necessary to organize a census beginning from the enrolment of the first member in the target group (generation) to the last one. Doing this may take many years. Of course this duration is not suitable for policymakers. Because of this difficulty, we must use statistical estimators as indicators.

To fix the ideas, let's consider now one cycle of school, with six levels of study and seven years as normal age for the enrolment. So, the Admission's Gross Rate (AGR), which is the usual approximation of the admission rate defined above, is:

$$AGR = \frac{\sum_{k=k_m}^{n} s_{1,t_o,k}}{p_{t_o,7}} \qquad (4)$$

Where, $S_{\boldsymbol{i,\,t,\,k}}$ is the number of children of $\boldsymbol{k}$ years old, enrolled during the school year $\boldsymbol{t}$, at level $\boldsymbol{i}$,. $\boldsymbol{K_m}$ being the youngest student's age. We can rewrite the $\boldsymbol{AGR}$ as following:

$$AGR = \frac{s_{1,t_o,k_n}}{p_{t_o,7}} + \frac{\sum_{\substack{k=k_m \\ k\neq k_n}}^{n} s_{1,t_o,k}}{p_{t_o,7}} \qquad (5)$$

$\boldsymbol{K_n}$, representing the normal age to access school.

It is clear that, the value of the second part of (5), corresponding to the number of students who are not members of the age group, can be very important. That is, this approximation is not the better one.

The legislation about the age of school access, being not always respected, notably in most African countries, one finds always the youngest and oldest pupil. Hence the following relation is true:

$$\boldsymbol{k_m < k_n < n}$$

The other estimator of the $\boldsymbol{AR}$ is the Admission's Net Rate (ANR) noted as follows:

$$ANR = \frac{s_{1,t_0,7}}{P_{t_0,7}} \qquad (6)$$

$$\Leftrightarrow \quad ANR = AGR - \frac{\sum_{\substack{k=k_m \\ k\neq 7}}^{n} s_{1,t_0,k}}{P_{t_0,7}} \qquad (7)$$

As we see, here the superfluous term is removed from the AGR. But, we don't take into account the members of the target group who came late into school, that is to say after the

normal age. This means that, the value of this estimator is still less than the number to be found.

Taking into account these two flaws, we propose another estimator, **generational admission rate** (***GAR).*** One writes it as follows:

$$GAR = \frac{\sum_{i=1}^{6} s_{i,t_o,7}}{p_{t_o,7}} + \frac{\sum_{k>7}^{n} s_{1,t_o,k}}{p_{t_o,7}} \qquad (8)$$

Here, we consider all members of the age group who are at school, at all levels (classes), as well as the pupils in the first year who have exceeded the required age. The second part of this relation is the estimated number of latecomers in the target group, those who registered after the school year t_0. We try to take into account all those who are not registered at the normal access age. So we reduce the biases existing with the usual estimators.

The difference between this indicator and the admission gross rate (***AGR***) is that part noted I_R.

$$GAR = AGR - I_R \qquad (9)$$

Where

$$I_R = \frac{\sum_{k<7} s_{1,t_o,k} - \sum_{i=2}^{6} s_{i,t_o,7}}{p_{t_o,7}} \qquad (10)$$

This part enables us to see the evolution of early entries, the children admitted before the required age.

$I_R > 0$, means that the number of children who have the preschool age continue to increase in primary school. In other words, there is a rejuvenation of the entire primary pupils. Thus

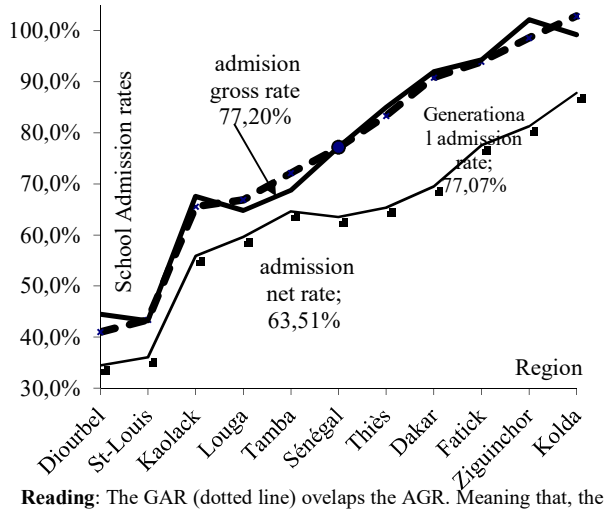
$$\mathbf{ANR < GAR < AGR} \qquad (11)$$

$I_R < 0$, means the reverse of the above phenomenon, which gives

$$\mathbf{ANR < AGR < GAR} \qquad (12)$$

Below (See Graphic II) we use the data of an African country [3,4] to illustrate this difference between the tree indicators.

Graphic II : School Access rates



Reading: The GAR (dotted line) ovelaps the AGR. Meaning that, the legislation is not well respected, there are 11 different ages (from less than 6 to over 14 years old) in the first year.

3. CONCLUSION

The usual indicators of school access, namely the *gross rate* or *net rate* of admission would give, in absence of good information about school, an idea of educational development in a country. But, by planning on this basis only, one runs the risk to miss the target.

The generational admission rate permits to better approximate the true measure of school access per generation; to know how the schooling grows in a society and to what extent the state respects its commitments to achieve quality education for all.

In addition to this, it is sufficient to have the distribution of schoolchildren by age to be able to calculate this indicator and deduce other useful information such as the recruitment policy.

As result, this indicator will help the policy maker or the planner to better appreciate the level of enrolment per generation and hence the schooling development. Add to this it characterize the policy of school recruitment. It shows how one fills the deficit about the request for access at the legal age. This can be done by the recruitment of children who have not yet got the required age or children who have exceeded this one.

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Analyzing the Associations between Educational Background Factors and Problem-Solving in Technology-Rich Environments: An investigation of United State Adult's Proficiency Level in PIAAC

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ABSTRACT

Examining interactive problem-solving tasks used by individual respondents and calculating the timing spent and the number of actions in each task helped us to present an exploratory study. This exploratory study used clustering and correlation analysis methods to prove the relationship between background skills such as learning, writing, reading, and ICT at home and at work. Specifically, using the public sample from the United States data, we drew on a set of process data collected from one problem-solving item and how that related to the background skills variables. Aimed research question: Are timing and number of action patterns related to problem-solving proficiency? What does each cluster have in common regarding their PS- behavioral patterns and background skills? In problem-solving, significant differences were founded among clusters based on the process data and the proficiency levels. The results implied that educational background skills related to PS-TRE process data and influential to the proficiency levels. These results will help us to understand the relationship between sequences of actions and proficiency estimates in large-scale assessments and held the promise of further improving the accuracy of problem-solving proficiency estimates. It will be possible to identifying factors associated with problem solving skills that can be of use in improving these competencies in U.S. adult education. Process data in log files will be analyzed to identify key factors associated with problem solving proficiency level and digital literacy. Additionally, patterns can be compared in problem-solving derived from a closer examination of the different strategies used by groups that vary in employment-related characteristics and the background education skills.

Keywords: OECD, PIAAC, ICT, Log-files, Process Data, large-scale assessment, Clustering analysis.

1. INTRODUCTION

BACKGROUND

In the last decade, a significant number of unique data have been collected on educational institutions and their internal processes and online systems with individual learners. The use of computers as an assessment delivery platform enables the development of new and innovative item types, such as interactive scenario-based items, and the collection of a broader range of information, including timing data and information about test-takers' processes engagement when completing assessment tasks [1]. Technical advances allow capturing timing and process data as test-takers solve digital problems in computer-based assessments [2]. Over the same period, educational data science with other multiple fields has arisen. Data science and engineering audiences were targeted for developing work in the field of educational data science. The focus began on applications such as computational psychometrics, machine learning applications for educational assessments and learning analytics. Greater competencies are needed to take advantage of new data science technologies. The Organization for Economic Co-operation and Development (OECD) sponsored a multi-cycle international program of assessment of adult skills and competencies such as the Programme for the International Assessment of Adult Competencies (PIAAC). The primary objectives of PIAAC are to 1) identify and measure cognitive competencies believed to underlie both personal and societal success, 2) assess the impact of these competencies on social and economic outcomes at individual and aggregate levels, 3) gauge the performance of education and training systems in generating required competencies, and 4) help to clarify the policy levers that could contribute to enhancing competencies [3].

RESEARCH OBJECTIVES

• This study addresses differential effects of time on task and number of actions on success across tasks used in the OECD Programme for the International Assessment of Adult Competencies (PIAAC).

• The study, also based on PIAAC data, investigates how the time spent on automatable subtasks during solving problems with respect to the entire task success.

• We investigate the relationship between the educational background skills and the number of actions taken during problem solving in PIAAC assessment.

• We study the impact of using Internet, communication and Technology (ICT) at home and at work in the proficiency level and score performance on Problem solving in technology-rich environments (PSTRE).

2. DATA: PROGRAM FOR THE INTERNATIONAL ASSESSMENT OF ADULT COMPETENCIES (PIAAC)

The Survey of Adult Skills (PIAAC) assesses the proficiency of adults from age 16 onwards in literacy, numeracy and problem solving in technology-rich environments. These skills are "key information-processing competencies" that are relevant to adults in many social contexts and work situations, and necessary for fully integrating and participating in the labor market, education and training, and social and civic life[4]. In Addition, the PIAAC survey collect information on the numeracy and literacy related to the respondent's activities such as the use of the educational background. Survey takers are asked whether their skills and qualifications match their work requirements and weather they have autonomy over key aspects of their work.

The data collected in log files, which represent information beyond response data (i.e., correct/incorrect), are incredibly valuable when examining interactive problem-solving tasks to identify the step-by-step problem-solving processes used by individual respondents. Such information is incredibly beneficial when examining interactive problem-solving tasks to determine the step-by-step problem-solving processes used by individual respondents [1].

As the largest and most innovative international assessment of adults, the Programme for the International Assessment of Adult Competencies (PIAAC), starting from the first cycle in 2012, has sought to assess computer, digital-learning, and problem-solving skills, which are essential in the 21st century [5]. Of significance here, PIAAC is the first international household survey of skills predominantly collected using information and communication technologies (ICT) in a core assessment domain: Problem Solving in Technology-Rich Environments (PSTRE). The PSTRE items that we focused on in this study were used to assess the skills required to solve problems for personal, work, and civic purposes by setting up appropriate goals and plans, as well as how individuals' access and make use of information through computers and networks [4]. The PSTRE domain section is not a measurement of "computer literacy." But instead of the cognitive skills required in the information age – an age in which the accessibility of boundless information has made it essential for people to decide what information they need, evaluate critically, and use to solve problems.

STUDY VARIABLES

There are standardized scales scores for skills use derived from items in the background survey. Index of Reading Skills at Home or Work (Non-nested scales) READHOME, READWORK: literacy skills for both document and prose type texts. Index of Writing Skills at Home or Work (Non-nested scales) WRITHOME, WRITWORK: writing skills such as writing letters, emails, articles, reports, or fill in forms. Index of Numeracy Skills at Home or Work (Non-nested scales) NUMHOME, NUMWORK: Numeracy activities such as calculation, prepare graphs/charts, use formulas, or advanced math. Index of ICT Skills at Home or Work. ICTHOME, ICTWORK: Literacy (Digital Reading), using computers and internet for various tasks (mail, documents). There are cognitive assessment scale scores for literacy test, numeracy test, and problem-solving test: First, Literacy - plausible values: PVLIT1 to PVLIT10. Second, Numeracy - plausible values: PVNUM1 to PVNUM10. And Problem Solving in Technology Rich Environment: PVPSL1 to PVPSL10.

3. RESEARCH METHODOLOGIES

A Couple of research studies cluster the participants in the assessment based on features extracted from process data and divide them into clusters that have in common regarding employment-related characteristics. The findings were that young, well-educated test takers with more work experience and higher work-related skills have higher chances of solving digital tasks. The higher-performing group is more likely to use actions demonstrating clear sub-goals, whereas the lower-performing group shows more frequent use of aimless actions [6].

DATA MINING APPROACH: CRISP-DM

A. K-MEANS CLUSTERING: We utilized the CRISP - Data Mining approach and considered the k-mean method used in multiple studies Scikit-learn K-means algorithm cluster data by iertia/within-cluster sum of squares. The definition of K-Means algorithm clusters data by trying to separate samples in n groups of equal variances, minimizing a criterion known as the inertia or within-cluster sum-of-squares. This algorithm requires the number of clusters to be specified. It scales well to many samples and has been used across a broad range of application areas in many different fields.

B. ELBOW METHOD The elbow method runs k-means clustering on the dataset for a range of values for k (say from 1-10) and then for each value of k computes an average score for all clusters. By default, the distortion score is computed, the sum of square distances from each point to its assigned center. The Elbow visualizer implements the "elbow" method to help data scientists select the optimal number of clusters by fitting the model with a range of values for k. If the line chart resembles an arm, then the "elbow" (the point of inflection on the curve) is a good indication that the underlying model fits best at that point. In the visualizer, "elbow" will be annotated with a dashed line.

C. EVALUATION METHOD: SILHOUETTE COEFFICIENT AND CALINSKI-HARABASZ INDEX (CH): The silhouette is a method used to interpret and validate the consistency within clusters of data. The technique provides a concise graphical representation of how well each object has been classified. The silhouette value is a measure of how similar an object is to its cluster compared to other clusters [7]. The Calinski-Harabasz index (CH) evaluates the cluster validity based on the average between- and within-cluster sum of squares [8].

4. RESULTS AND DISCUSSION REMARKS

VISUALIZATION AND DESCRIPTIVE STATISTICS

After determining the targeted variables and factors that we are going to carry on for the rest of the study, frequency plots of Gender, Education Level as main variables were plotted.

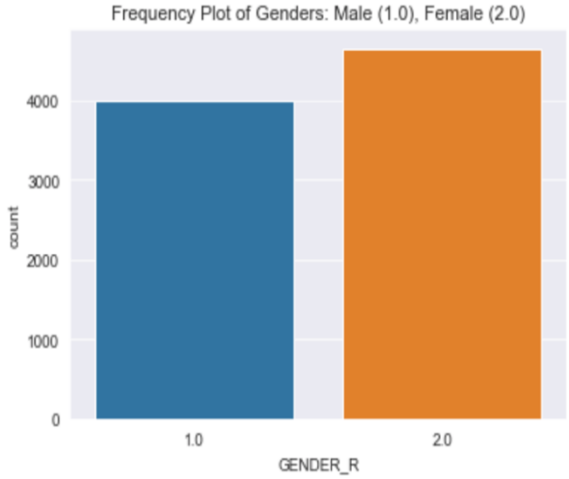

Figure 1: Frequency Plots for gender

For Plot (GENDER_R) Result showed 1.0 = ~ 4000 Male, 2.0 - Female =~ 5000. The second plot present the highest education level by the International Standard Classification of education (ISCED). 2: Grade 1-6; 3: Grade 7-9; 7: High School Diploma; 9: Cert. from trade school; 11: Associate Degree; 12: Bachelor's degree; 13: Master's degree/Prof. Degree; 14: Doctorate Degree; 15: Foreign Qual. Highest number of participants in the survey is high school diploma graduates while the lowest participants are Ph.Ds.

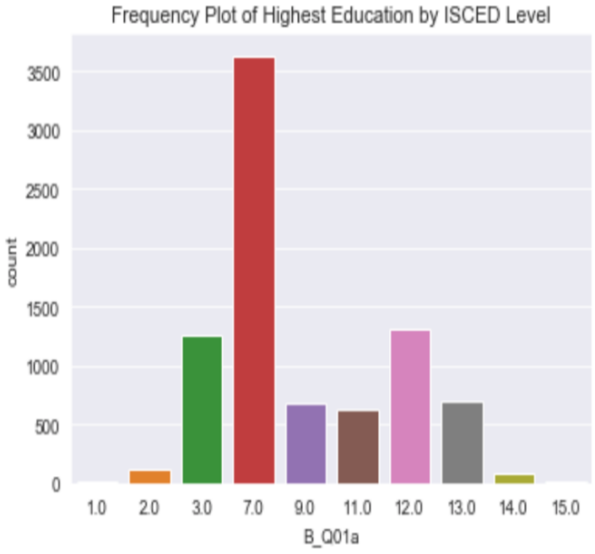

Figure 2: Frequency Plots for Education Level.

Secondly, a distribution of index variables helps to describe the background for the assessment takers. As weighted amounts of interacting with numeracy, literacy and ICT at home and at work.

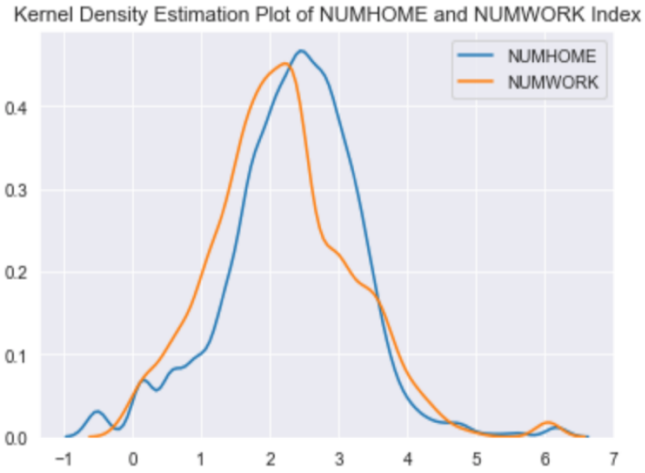

Figure 3: Kernel Density Estimation plots for numercy at home and at work.

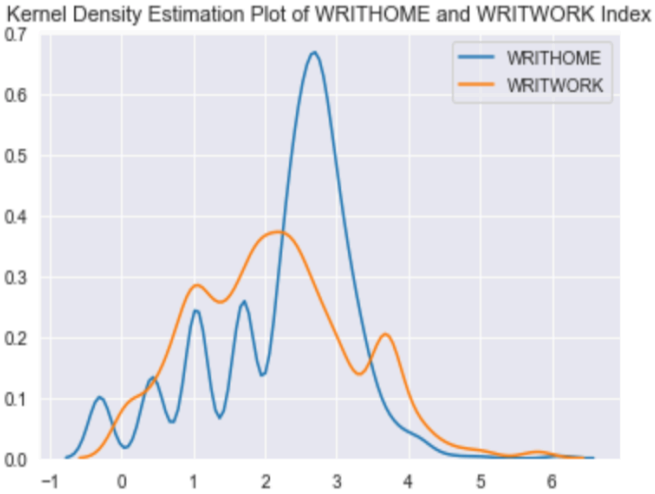

Figure 4: Kernel Density Estimation Plots for Writing at Home and at Work.

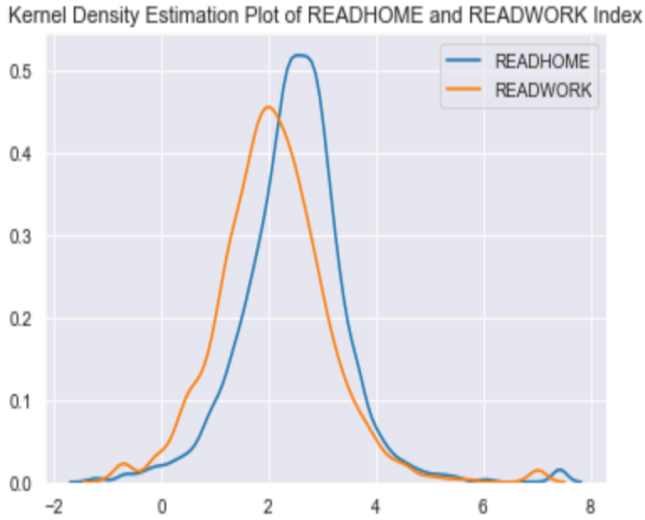

Figure 5: Kernel Density Estimation plots for Reading at Home and at Work

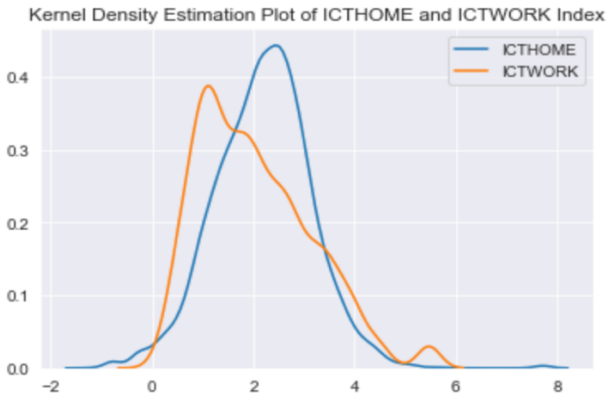

Figure 6: Kernel Density Estimation plots for ICT at home and at work.

Figure 7: Box and Whisker plot of Index in background Variables.

CLUSTERING K-MEANS ANALYSIS

By utilizing the CRISP-Data Mining approach and considering the k-mean method, we will be able to subset the demographic and background variables. On the other side, the index variables and add each set of plausible values of problem solving in technology environment. The first step we use the Elbow method to determine the Optimal k Value. The value of k and the some of the square error suggested 4 clusters.

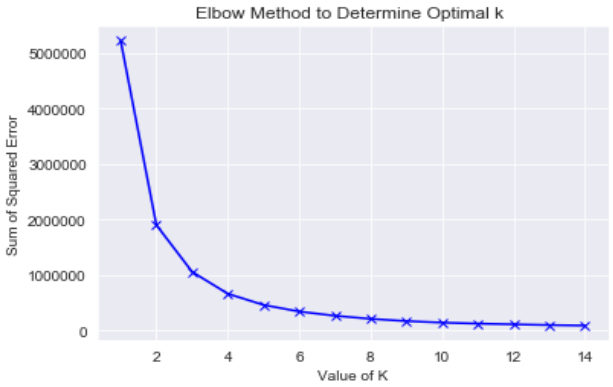

Figure 8: Elbow methods determine the number clusters.

Evaluation	Silhouette Coefficient	CH Index
Model 1 (5 Clusters)	0.494	8131.6120
Model 1 (4 Clusters)	0.503	7212.569
Model 1 (4 Clusters) Reduced Variables	0.5163	6270.902
Model 2 (4 Clusters)	0.2928	3210

Table 1: Evaluation Method Results to Validate the Clusters

In model one, we explored the possibility of removing index that are not reliable indicators of the person's characteristics. However, the result did not appear to improve much on the performance. Another way to explore the background variables and the assessment item score is to look at each cognitive domain separately. In model 2, We separate the cognitive assessment scores and add only the numeracy set to the index. Then, we continue with 4 clusters k-mean analysis.

Review Results and Determine Characteristics of Clusters

Here, we will review the cluster membership based on the 4 cluster solutions with background variables & one set of the cognitive assessment scores (PVPSL1).

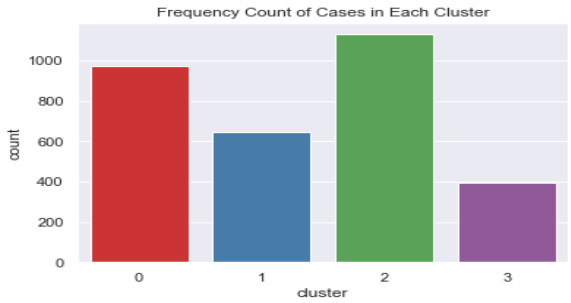

Figure 9: Frequency count in each cluster.

Appending and merging demographic variables, and cognitive assessment score for the (PSRET) to show the characteristic of each cluster.

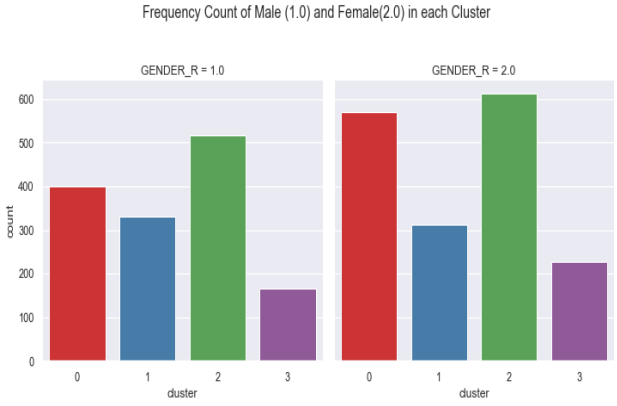

Figure 10: Categorical Plot of gender variable by categories.

The categorical plot of gender presents the frequency count of males and females in each of the decided clusters. It is obvious that females were the majority taken the assessments. Both clusters 0 and 3 have the majority of the males and females.

(N = 3136)			
Cluster 0	**Cluster 1**	**Cluster 2**	**Cluster 3**
N = 971	N = 643	N = 1130	N = 392
Female	Male	Female	Female
HS Diploma	Bachelor's degree	HS Diploma & Bachelor's degree	HS Diploma
ICTHOME: 2.22	ICTHOME: 2.70	ICTHOME: 2.52	ICTHOME: 2.09
ICTWORK: 2.04	ICTWORK: 2.46	ICTWORK: 2.32	ICTWORK: 1.85
PVPS1: 261.04	PVPS1: 338.36	PVPS1:299.03	PVPS1:214.51

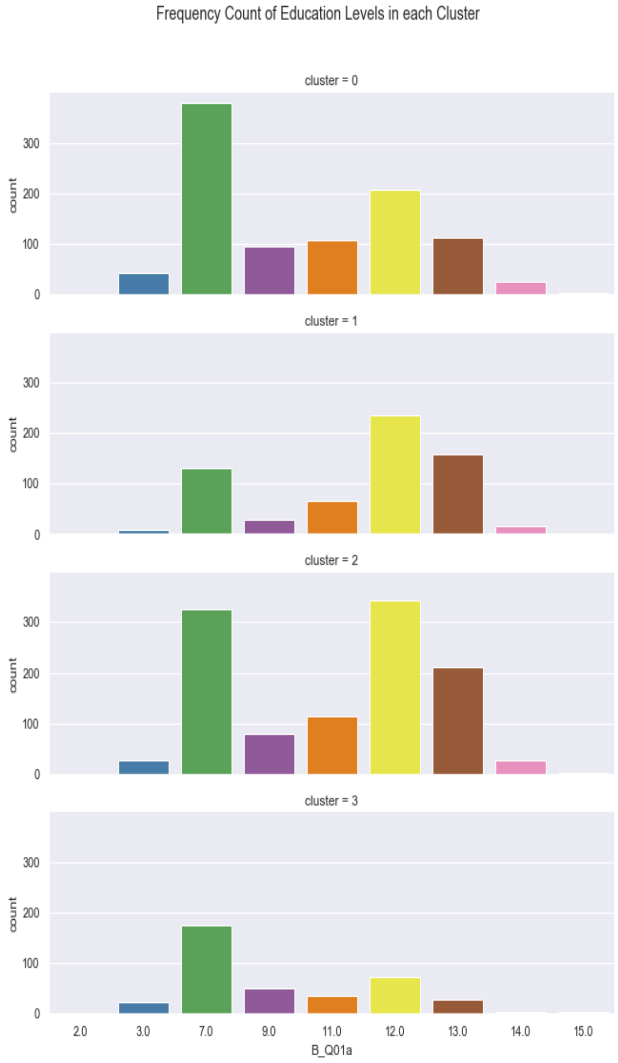

Figure 11: Categorial Plot of Education Level by Clusters Displayed in Subplots

The distribution shows the count of highest degree gained by participants in each cluster. Ph.D.'s and masters are the least in each cluster which proves the small number of participations over all in this assessment. All the clusters except cluster one appears to have high school diploma the majority. Bachelor's degree holders are the majority in both clusters 1 and 2.

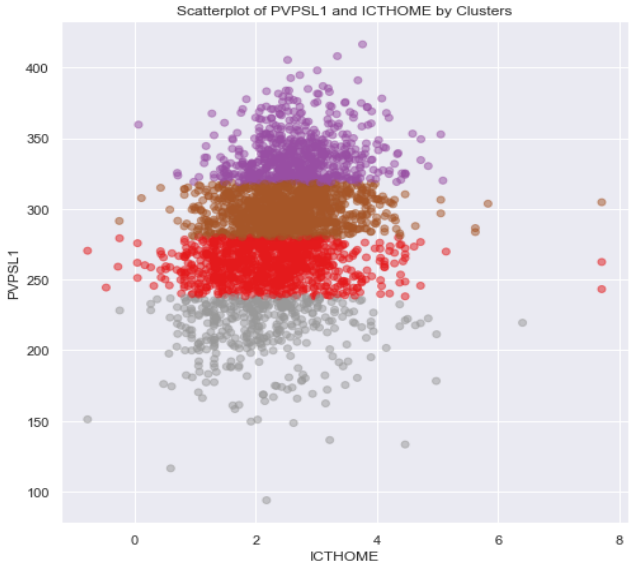

Figure 12: Scatterplot of Clusters by the Index Variables (PVPSL1 and ICT Home)

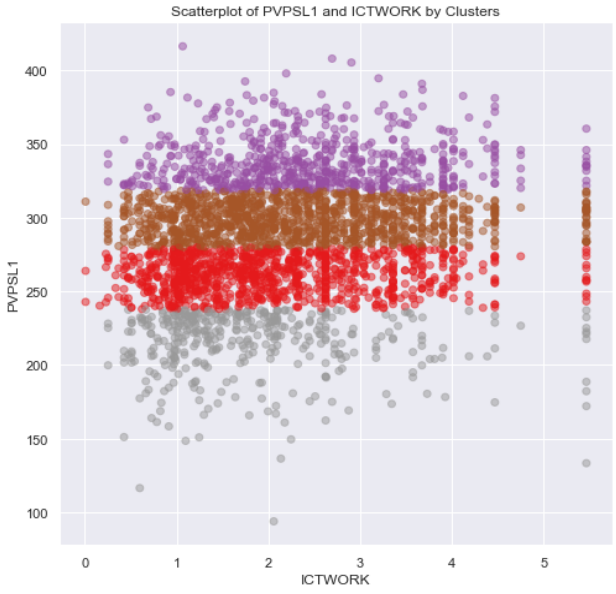

Figure 13: Scatterplot of clusters by the index variables (PVPSL1 and ICT Work)

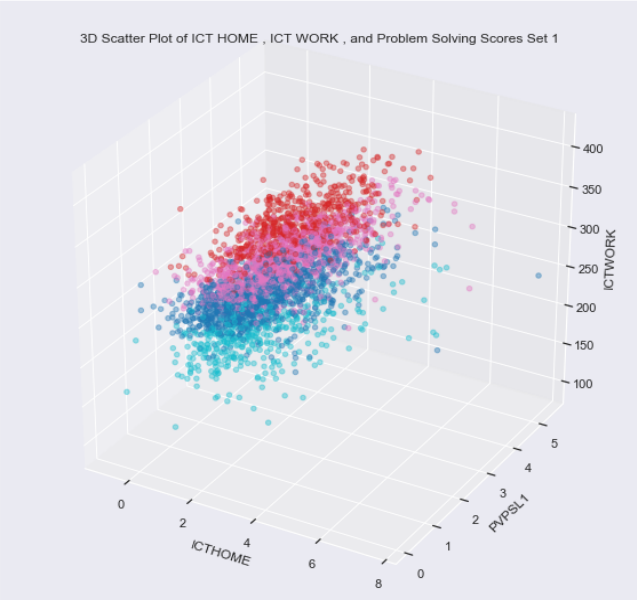

Figure 14: 3D Scatterplot of Clusters of Variable Factors (ICT Home and Work / Problem Solving Score set 1)

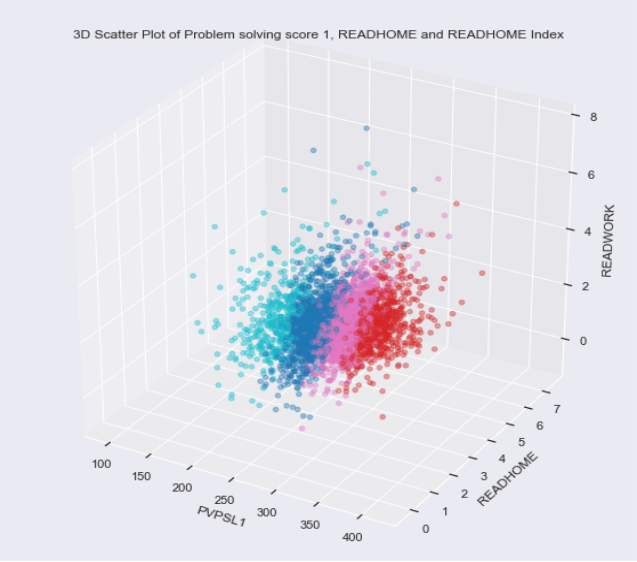

Figure 15: 3D Scatterplot of clusters of variable factors (Reading Home and Work / Problem Solving Score set1)

5. CONCLUSION AND FUTURE WORK

PIAAC has many unique aspects; one of them is that of the computer-based assessment with adaptive design within literacy and numeracy domains. The adaptive testing process works by directing respondents to a set of easier or more difficult tasks based on their answers to the core questions and literacy/numeracy core questions, which are automatically scored as correct or incorrect. A model will be used to determine which participant mainly will be tested in certain assessment items based a set of variables that includes a) the language native or non-native participant speaker, b) the level of education; and c) how is the participant performance in the CBA Core. There is no adaptive process for the problem-solving domain.

Creating an adaptive design model for the problem-solving assessment domain will provide a key advantage to more accurate assessment. Traditional test design in which respondents must answer all questions included in the test, from easiest to most difficult. That design did not accurately present the proficiency level score for each participant. Using a smaller number of assessment items will better determine participants' abilities.

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Modeling Organizational Structure of Scholarship of Teaching and Learning Program: Transition from Consumers to Producers of Knowledge

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ABSTRACT

Similar to a community-of-practice, the members of Scholarship of Teaching and Learning (SoTL), an institutional infrastructure, are driven by a shared interest and enthusiasm to improve their teaching and learning. Representing time-varying SoTL events and relationships between SoTL members as a community network introduces challenges in data linking, data model, and network analysis. In particular, it is essential to design solutions to preserve the network topology, temporal information, member status transformation, and diverse relationships between nodes. In order to account for the SoTL network complexity, we design a heterogenous graph model in the Neo4j graph database. The graph database offers a novel research method to the growing interdisciplinary SoTL field. This paper will describe the model design, challenges, and network analysis to evaluate the effectiveness of the current SoTL strategies in attracting new members and supporting the sustainability of existing cohorts and provide data-driven decision support for SoTL programs in their development and priorities.

Keywords: Scholarship of Teaching and Learning, social network, neo4j.

1. INTRODUCTION

In the last few decades there has been a proliferation of institutional initiatives to promote faculty excellence and innovation in teaching and learning. Faculty development activities range from traditional programs (e.g., workshops, seminars, short courses, fellowships, conferences) to alternative approaches, such as self-directed learning, mentoring, peer-coaching [1]. Among the top-down and bottom-up approaches, the teaching innovation is effectively shown with "a participatory, collaborative methods to identify problems and solutions" and sharing leadership among all stakeholders [2, p.29]. From an organizational perspective, faculty development has been conceptualized as a taxonomy with three levels of engagement: good teaching, scholarly teaching, and the scholarship in teaching and learning [3]. Good teaching can be described as a practice, scholarly teaching is a practice of a teacher engaged with scholarly literature, whereas the scholarship in teaching and learning is conceptualized as a community of practice (see Figure 1).

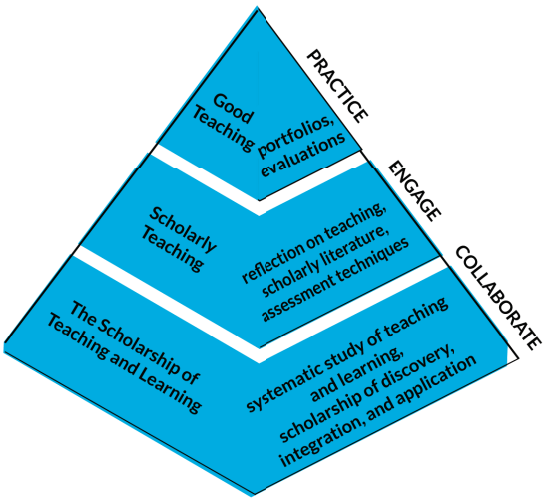

Figure 1: The taxonomy of faculty development from a single practitioner toward a community of practice (adapted from [3]).

Teaching taxonomy can also be represented on a two-dimensional plane, as suggested in the Dimensions of Activities Related to Teaching (DART) model [4]. The DART classification places teaching activities on a continuum from private to public and from systematic to informal: good teaching is positioned in the lower left quadrant (private and informal), scholarly teaching is on the top left quadrant (private and systematic), sharing about teaching activities is positioned in the bottom right quadrant (informal and public) and scholarship of teaching and learning is located in the top right quadrant (systematic and public), as illustrated in Figure 2.

Since its origin [5], the term *Scholarship in Teaching and Learning* (SoTL) has evolved into a complex multidisciplinary institutional infrastructure ensuring the support of research related to teaching and learning and high quality in education on three levels: micro-social (individual educators), meso-social (collaboration between educators), and macro-social (institutional policies) [6]. Several factors have been attributed to successful integration of SoTL into institutional culture: institutional support (funding and fellowship), departmental support (encouraging climate), collegial interaction (discussions, teams), professional development

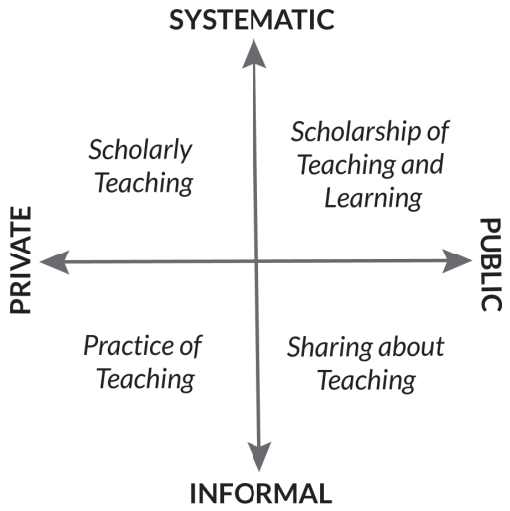

Figure 2: The DART model organizes teaching-related activities as a two-dimensional plane with four quadrants: from private to public and from systematic to informal [4, p.5].

opportunities (workshops and seminars) [7, 8, 9]. The departmental support has been identified as one of the strongest institutional motivators for individuals to become engaged into a SoTL inquiry [10, 11, 12]. Within the department, Roy et al. identified three key roles facilitating the SoTL integration: 1) initiators with strong ties or in the position to bring changes, 2) implementers or participators in changes, and 3) cultivators or creators of groundwork [11]. The sustainability of SoTL research must be cultivated "by nurturing significant networks" at the institutional level [13, p.59]. In this view, SoTL community can be perceived as a community-of-practice (CoP) driven by shared interests and enthusiasm to improve their teaching and learning. Within each community, members share characteristics of social networks, where individuals build strong or weak connections, influence other members or become isolates, create bridges between communities, grow community or disappear over time [14].

In this paper, we will examine the formation, practice and evolution of SoTL communities at Indiana University, a research institution which celebrated the 20th anniversary of the SoTL program. The study of the SoTL communities as network offers a novel and promising approach to 1) evaluate the effectiveness of current SoTL strategies in attracting new members and supporting the sustainability of existing cohorts, 2) provide data-driven decision support for SoTL program development and priorities, and 3) identify patterns of the internal structure of CoPs. The second aim is to gain insights from the internal structures of these network communities and subsequently identify SoTL influencers, active members, who help diffuse the knowledge and awareness about SoTL among their peers. Of particular interest is whether there exists a transition pattern from passive members into active influencers. This information will have a practical contribution to SoTL program and will be used to obtain qualitative data via interviews with influencers about SoTL experiences in the second stage of this research. Finally, we will look at the co-authorship network using the publication database by SoTL members. Scholarly publication metrics can be further used to measure the academic return of investment (ROI), whereas the financial ROI is represented by SoTL funding [15]. The ROI insights are essential on a meso-social level for measuring financial and academic impact of the SoTL program and securing institutional support.

The remainder of this article is structured as follows: Section 2 will summarize the characteristics of SoTL communities, the graph design and dataset are outlined in Section 3. Section 4 presents the results of network analysis and Section 5 concludes with future direction of research.

2. BACKGROUND: SOTL SOCIAL NETWORKS

Faculty tend to become aware of SoTL and learn to do SoTL through networking with peers. This organic learning is often referred to as "personal learning networks" - social communities that provide their members with guidance to advance skills and knowledge in a particular area via the benefit of collective knowledge as well as create the network of connections. These networks have been shown to be more effective at enhancing teaching than more traditional models of professional development [16]. This is unsurprising given that experienced faculty members tend to have larger personal learning networks than junior faculty [17]. Recent research also suggest that faculty development programs could serve as mediators to connect members [18]. For example, CoPs are created from the Faculty Learning Communities organized by Centers of Innovative Teaching and Learning (CITL) and CITL consultants/mentors embedded within CoPs connect across members other communities [19, 20]. On the other hand, isolation has been identified as one of the most challenging barriers facing SoTL [9]. Thus, it becomes crucial to develop "a critical mass of SoTL champions" who will create and maintain social networking, help diffuse ideas and sustain SoTL communities [9, p.53].

SoTL communities are viewed as small tightly bonded networks with a few members that can connect between network communities [9]. Each member can participate in a variety of SoTL events: keynote speakers series, book reading groups, faculty learning communities, grant initiatives, and co-authored publications. While participation in some events is passive (a.k.a attendance), other events require active involvement (e.g., writing and collaboration). Using the DART model (see Figure 2), active involvement can be further described as a private-public and informal-systematic continuum [4].

The following schema represents the DART placement for research related activities on a continuum from less systematic to more systematic and from less public to more public: 1) Systematic: published case studies/published essays < literature review < textbook < meta-analysis < peer-reviewed presented or published research, and 2) Public: published case studies < published essay/literature review < meta-analysis < peer-reviewed research < textbook.

SoTL membership often starts with a passive involvement via attending professional workshops or seminars, where new members become aware of SoTL initiatives and networks. At this stage, members are 'consumers' of shared knowledge. This status may subsequently change to a 'producer' if they get involved in a scholarly SoTL research via funding, fellowships, and publishing. In fact, producers are the main driving force for public dissemination and continuity of shared knowledge and innovations. They are also in a position to become strong links within SoTL networks. We expect these individuals carry similar characteristics of strong nodes in network. For example, they have many connections, bridge between disciplines or programs, and have influential publications (measured by the number of citations they generate). Social network design makes it possible to explore the temporal trajectory of those "influencers" from a consumer to a producer, their characteristics, collaboration with others, and scholarly work.

3. DATA AND METHODS

Current SoTL SQL database contains 6 tables covering the period between 2011 and 2019: Funded SoTL Projects (64 records), SoTL Funded/non-Funded Proposal (102 records), Reading Groups (65 records), SoTL Event Attendance (513 records), Faculty Learning Communities (19 records) and SoTL Faculty List (142 records). The publication data (680 records) is extracted from the web server and provides publication records starting from 1974, as these records also include scholarly teaching and sharing activities prior the conceptualization of the scholarship of teaching and learning field. One of the main challenges in this dataset is the absence of usernames in publication dataset. Second, the co-author can be a graduate student, faculty outside of IU, faculty who left IU or a consultant from Center for Innovative Teaching and Learning (CITL). If the paper has multiple co-authors, we identified a username only for the first author or a faculty author from IU by searching IU web faculty lists (115 unique names). Note that if the faculty left the institution, we were unable to identify their username. For the SoTL graph model, we use only the 2011-2019 period (115 records) whereas the full set (680 records) is used for a co-authorship network. Next, 7 tables are merged into one CSV file with the total of 785 records. While each dataset has a username field, the merger on username created null values, since we used a join outer merger. In addition, we added ranking values for each events and calculated the total number of events attended per SoTL member. Our next step is to convert the relational database (a set of tables with rows and columns) into a graph database where data is stored as a network with nodes and edges (links). Table 1 provides a summary of variables that will be used to build the network schema.

In our approach, we design a SoTL network based on the following functionalities similar to the Content-Filter recommendation approaches: 1) membership - there is a unique account for each user, 2) communities - each user belong to one or more groups (workshops, reading groups, faculty learning communities), 3) product - each user can produce a scholarly item (grant proposal, publication), 4) collaboration - each user can interact with others producing scholarly items, 5) rating - each user is rated on the scale 1-5 based on the commitment with 5 being the highest rating (Workshops - 1; Reading groups - 2; Faculty Learning Communities (FLC) - 3; SoTL proposal - 4; SoTL Publication - 5). We included an additional weight variable for publication based on the publication format: Visualization/Other -1, Report -2, Poster - 3, Presentation - 4, Journal Article/Conference paper/Book/Book Chapter - 5. The graph model design is presented in Figure 3. SoTL members and events are mapped into nodes with a variety of properties. The states of attending, submitting and producing (a.k.a publishing) form edges. We have also dedicated a node for a school which could be used to measure interdisciplinarity and collaboration. One of the issues working with the merged dataset was null values. In our current solution, we added a new value "Unspecified" or FALSE or zero as well as created unique indices for each variable that will be a node even if the value is null (e.g. publications, events, proposals).

To store the SoTL network model and query across nodes and edges, we chose Neo4j [21], an open source graph database, which is flexible and particularly suited for heterogeneous datasets [22]. With the defined schema, 7 nodes are created in Neo4j and uniqueness constraints are placed on usernames and on each event ID using Neo4j Cypher plugin. We created 6 relationships with the rank properties, which would allow us to specify a rank (from less commitment to more commitment) while querying for events attended. As a result, our network has 4,490 nodes and 4,710 relationships.

To gain insights on the internal structure of SoTL communities and their characteristics we will perform network and co-authorship analyses. The network analysis is performed using SoTL graph database and APOC Cypher plugin. Particularly, we aim to examine whether there exist any similarities between "consumers" (attending only) and "producers" (publishing). First, we retrieve their characteristics and search for any members who changed their states from a consumer to a producer. Second, we use Neo4j Data Science Graph library and

Table 1: SoTL datasets summary of variables for Network Model

Dataset	Variables
Funded SoTL Projects	Project ID (unique), User name (PI), Amount, Year
SoTL Funded/non-Funded Proposal	User name (PI), Year, Funded (boolean), Rank
Reading Groups	User name, First year Joined, Total of groups joined, Rank
SoTL Event Attendance	User name, Total keynote speakers attended, Rank
Faculty Learning Communities (FLC)	User name, Total FLC attended, Rank
SoTL Faculty List	User name, School, Rank, Fellowship (boolean)
Publication data	User name, Format, Rank, Discipline, Year

Note: Fellowship status can include Mosaic, Learning Analytics or Mack fellowship.

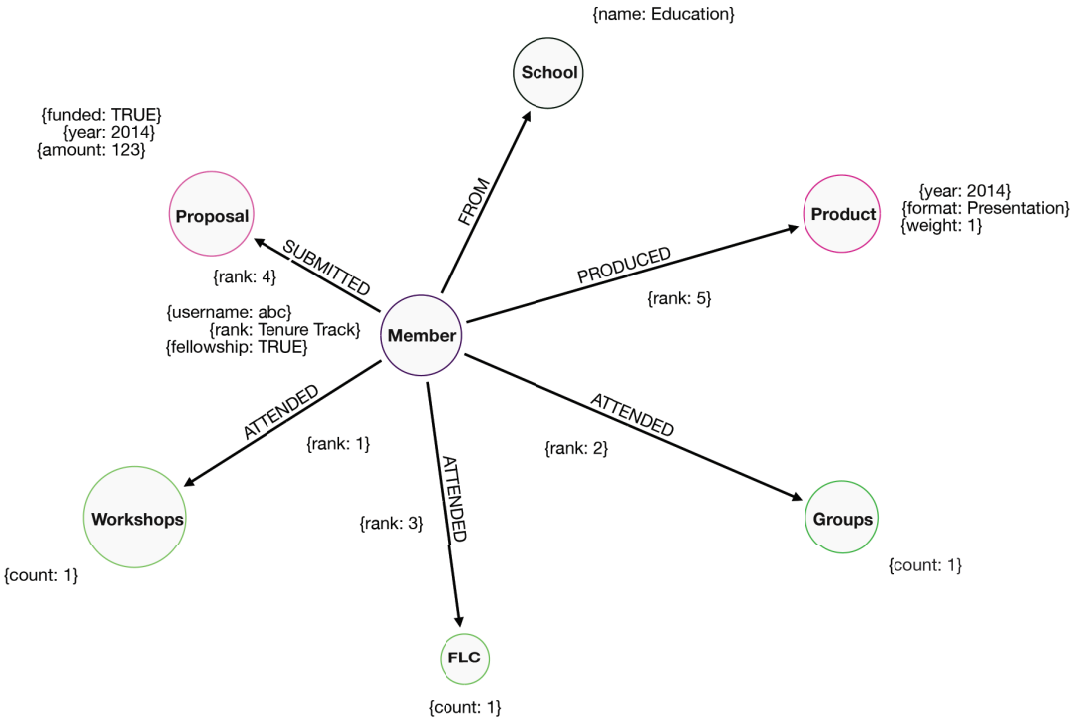

Figure 3: Neo4j Model of SoTL network. Members and events are represented as nodes. The relationships between nodes are shown as edges. Node and Edge attributes are enclosed into curly brackets.

apply Jaccard algorithm to measure similarities within members [23].

Co-authorship network analysis is performed using Sci2 [24] and Gephi [25]. The publication data is pre-processed merging all co-authors into one column while separating them with "|". At this stage, we used only Authors names information. Sci2 has a functionality to generate a co-occurrence network which is then exported into a graph xml format.

4. RESULTS AND DISCUSSION

We used the Sci2 Tool to extract a co-author network and network analysis toolkit to examine network components [24]. The undirected weighted network consists of nodes representing a unique author and edges showing authors co-occurrences. The resulting co-author network has 297 nodes, 11 isolates (nodes that do not connect to other authors) and 573 edges. After removing 11 isolates using Sci2 built-in feature, we obtained the network with 286 nodes and 573 edges. The ForceAtlas2 layout with Noverlap was applied to graph data and visualized with Gephi [25] (see Figure 4).

The co-authorship network structure exhibits several types of co-networks: 1) a small network with one influential node (George Kuh), 2) a larger dense network with several mid-size nodes and strong connections, and 3) small networks. These patterns point to several initial hypotheses that will be tested in the second stage of this research. First, a dense network seems to be time contingent. Several large nodes represent faculty members who started at the same time and seem to continue working together. Secondly, several nodes are represented by Center for Innovative Teaching and Learning consultants. As nodes, they connect with faculty, however they are not

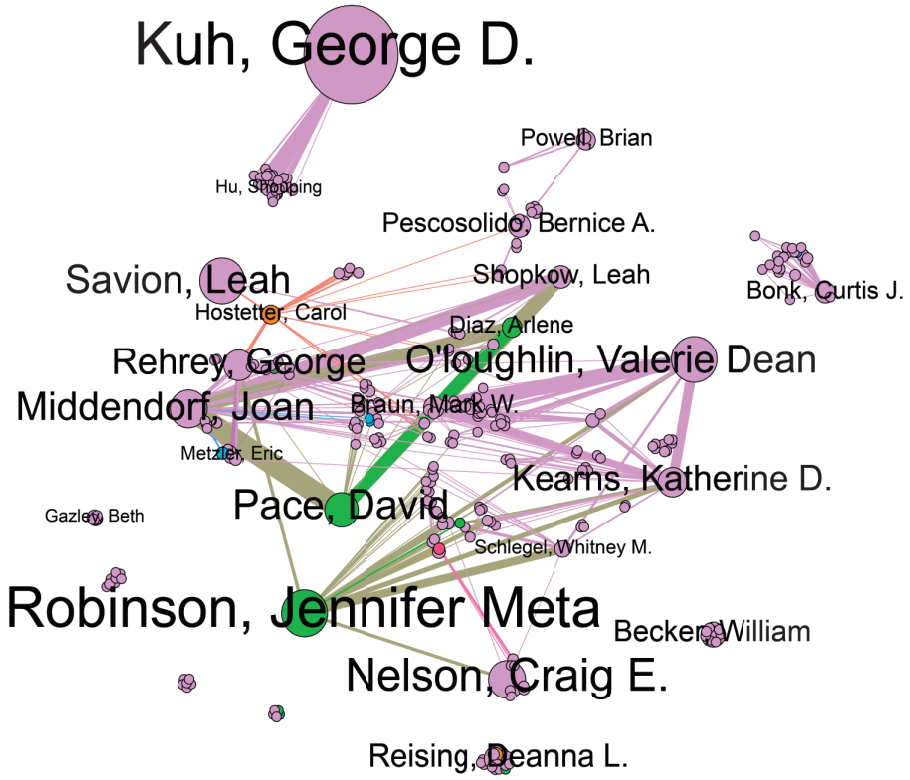

Figure 4: Co-authorship network. The size of nodes represents the number of scholarly work with the largest node = 147 and the smallest node =1. The largest component consists of 157 nodes. The labels are shown only for authors with 10 or more publications. The thickness of edges indicate the number of co-publications. The node color indicates authors' school: purple - unidentified (authors were not in the current SoTL database), green - College of Arts and Science, brown - Social Work, red - Public Health, blue - Informatics.

connected to each other, which suggests that they may be important connection points for faculty work. Third, a large influential node in a smaller cluster is a former SoTL program director, suggesting a key position for creating and sustaining SoTL networks. Finally, there are many isolated nodes, leading to a strategic question on what initiatives need to be implemented to connect them with a larger network.

We have also designed an interactive co-authorship network, available at https://obscrivn.github.io/SOTL/. The visualization is built using JavaScript GEXF Viewer for Gephi [26]. This platform offers several practical functionalities for SoTL researchers who might be searching for collaborators or exploring published research on teaching and learning, e.g., authors information (school, discipline, rank, publication counts), search options, and links information.

Our initial hypothesis is the existence of two types of membership (consumers and producers), where consumers may transition to producers after learning about SoTL via informal events. Using the count property [count $>$ 0] for FLC/Reading Group/Workshops, funded property [funded = 'TRUE'] and weight for publications [weight $>$ 0], we identified only two members who attended SoTL events, submitted a proposal and also published scholarly work (see Figure 5). Most of the membership falls into 1) consumer only - 71 members, 2) producers only - 49 members. In the producer category, 13 members have a fellowship. However, the consumer group has only 1 member with a fellowship, suggesting that fellowship is an important institutional initiative that can lead to publications and proposals.

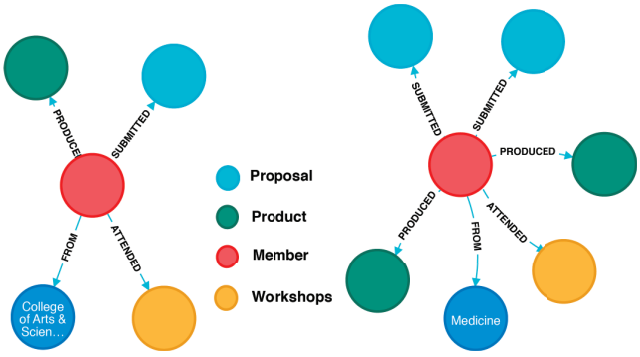

Figure 5: SoTL members who are consumers (attended events: rank 1-3) and producers (submitted proposal and published: rank 4-5

On the departmental level, we examined 7

communities. Note we have a large "Unspecified" value for missing entries. These values will be manually entered in the next stage. The current top three schools are College of Art and Sciences include 39 SoTL members, Education - 34, Public Health - 19. These information can be used externally to help new faculty connect with their SoTL departmental CoPs.

Next, we examined the faculty development events (workshops, groups, and FLC). The most attended event is workshops series (300), followed by Reading groups (65) and FLC (19). This finding supports our proposed taxonomy from less commitment to more commitment and if we include fellowships (14), the SoTL taxonomy can be represented as a 6 level scale: Workshops $<$ Reading groups $<$ Faculty Learning Communities (FLC) $<$ Fellowship $<$ SoTL Proposal $<$ SoTL Publication.

To measure the similarities, we applied the Jaccard algorithm to all members including all relationships. For this query, we replaced the previously assigned three types of relationships (ATTENDED, SUBMITTED, PRODUCED) to just one - LINK with a rank property that identifies the type of event (workshops - 1, reading groups - 2, FLC - 3, proposals - 4, publication - 5). The Jaccard algorithm is implemented as follows [27]:

```
MATCH (p1:Member)-[:LINK]->(m)
WITH p1, collect(id(m)) AS p1m
MATCH (p2:Member)-[:LINK]->(m2)
WITH p1, p1m, p2, collect(id(m2))
AS p2m
RETURN
p1.name AS from,
p2.name AS to,
gds.alpha.similarity.jaccard(p1m, p2m)
AS similarity
```

SoTL members as individuals do not show any similarities in the choices of events, suggesting that each faculty member has their own trajectory based on the current teaching needs. It also points to the weakness of our current model where events are not interconnected.

5. CONCLUSION AND FUTURE DIRECTIONS

This paper extended the recent work on SoTL as a Social Network [9]. We used a co-authorship network to identify influencers playing a key role for SoTL dissemination and sustainability. The co-authorship network analysis demonstrated the existence of several dense isolates clusters. Our recommendation to SOTL program is to identify strong links via our co-authorship network and develop initiatives connecting those leaders. Second, we contributed to the SoTL field by introducing a novel Neo4j graph database approach to explore the SoTL characteristics. The graph model allowed us to confirm our hypothesis on the existence of two subgroups: consumers and producers, with only two members who transitioned from consumers to producers. That is, attending informal events (passive involvement) does not necessarily lead to proposal and publication (active involvement). However, we found that fellowship initiatives seem to encourage scholarly work dissemination, as it was one of the main distinctions between consumers and producers based on our data. We suggest that the SoTL program increase the outreach initiatives to promote the awareness fellowships. We also propose to develop a collaboration recommendation system helping connect weak links with possible strong connections and allowing new members to become actively engaged in collaborative work based on their shared interests. Finally, SoTL consultants should be viewed as core influencers building CoPS and identifying strong leaders from faculty to sustain those communities [18].

At present, the neo4j model is centered on SoTL members and their individual activities. In particular, this model does not account for event sequences and event networks. Future work should examine event dependencies and analyze clusters within and between each event. Similarly, the current model connects members only via schools. Future model should interlink members via event, shared interest, and their collaboration (presentations, proposals, publications) allowing for a more complex network analysis of SoTL communities. Furthermore, the co-authorship network model could be enriched with additional attributes: 1) node level features (e.g., number of citations, publications), 2) multiplex level relationships (e.g., co-authoring and supervising students), and 3) cognitive network (e.g., similarity between papers). These attributes would help us examine the role of mentorship and team-based collaboration. Finally, the results from the social network analysis should be used for a qualitative analysis of SoTL influencers, thus contributing to the field with a mixed-method social network approach.

6. ACKNOWLEDGMENT

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Power Grid Vulnerability Analysis with Rising Renewables Infiltration

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ABSTRACT

The increasing penetration of renewable energy has a significant impact on the performance and reliability of the power grid. This is largely because of the uncertainty of the renewable resources and the complex nature of the power system infrastructure. This paper analyzed power grids' vulnerability to cascading failures with respect to the penetration level of renewable energy into the grid. In this paper, a novel power balance technique is used for cascading failure analysis and power grid vulnerability measurement. The proposed approach incorporates a modified optimal power flow algorithm in the grid vulnerability analysis study which accurately reflects the most probable path of cascading failure evolution process with uncertain renewable generation. The simulation results on IEEE 118 bus system and IEEE 300 bus system showed that increasing penetration of renewable energy have proportionally higher impact on grid vulnerability to cascading failures due to injection of higher uncertainties into the grid.

Keywords: Cascading Failure, AC Power Flow, Optimal Power Flow, Grid Vulnerability, Uncertainty.

1. INTRODUCTION

The modern power systems are undergoing some massive transitions. Growing demand of electricity, when combined with the need to limit carbon emissions to achieve sustainable development goals [1], encourages a rapid growth of renewables into the existing power system. However, the unpredictable nature of renewable sources will inject significant amount of uncertainty into the grid which may lead to cascading process resulting into blackout. So, it is necessary to study the impact of renewable energy (RE) penetration to grid vulnerability analysis to cascading failures (CF).

The literature on CF mainly focuses on the modelling and analytical tools for a given network. There are two types of models in simulating CF; transient models and steady state models. In [2], a dynamic model is presented which can simulate various cascading outage mechanisms. Steady state models are more common in literature to study and evaluate CF process. In [3], [4], the mixed OPF-stochastic models are the examples of steady state CF model where DC power flow (DCPF) was used to reduce computational burden. As DC models resulted in large errors in flow estimation [5] for large networks, [6], [7], are among the models incorporates AC power flow in the simulation of CF. In the stochastic CF model in [7], conventional optimal power flow (OPF) is used in the power balance algorithm which failed to accurately evaluate the negative impacts introduced by RE on the grid vulnerability.

In our proposed model, a new CF model with AC power flow (ACPF) method is used for vulnerability analysis of the grid network to cascading process. Cascading failure is a fast-evolving process. So rather than using conventional OPF, the proposed approach incorporates a modified OPF algorithm where least square adjustment method is used to restore the balance in the network during cascading failures and in result, the simulation process accurately mimics the most probable evolution path of CF process. To measure the vulnerability to CF, load shedding amount and number of tripped lines for increasing RE penetration are used as measuring tool in this study. Simulations have been performed on IEEE 118 bus system and IEEE 300 bus system for different testing cases and scenarios. This study has also revealed that after a certain level of RE penetration, system may deteriorate rapidly and preventive measures should be taken if and when RE penetration level exceeds that limit in order to mitigate the negative impacts of RE penetration during CF process.

The rest of the paper is arranged in following parts. In section 2, the system model is briefly discussed. Section 3 contains the modified OPF algorithm and the overall simulation workflow. Section 4 and 5 present results and conclusion, respectively.

2. SYSTEM MODEL

The modern power system is an ever-evolving complex infrastructure. Their complex interconnected nature along with characteristics of different parameters introduce uncertainties into the network. The goal of this study is to assess the grid vulnerability to cascading process from uncertainty perspective. Our proposed model considers uncertainty from load and renewable generation which propagates linearly in the line flow process.

A. Uncertainty modelling

In the proposed uncertainty model [8], the load demand power and renewable generator output power are represented with two terms as follows:

$$P(t) = \mu_p(t) + \epsilon_p(t) \tag{1}$$

where $\mu_P(t)$ is the mean of load demand power or renewable generator output power at time *t*. In other words, it is the expected power signal ahead of time. It is actually the forecasted power we achieve through some forecasting techniques using historical data. And $\epsilon_P(t)$ represents the uncertainty which is a zero-mean signal. It is the difference between forecasted data and actual data. In this study, we assume load forecasting errors and wind output power mismatches as uncertainties. As the historical data and future data follow same pattern for load demand and renewable resources, a widely popular forecasting model, Autoregressive Moving Average (ARMA) technique [9] is used to model uncertainties in this study.

B. Line flow based on AC power flow

The power flow study is the numerical computation of voltage magnitude and phase angle at each bus in an interconnected network under steady state condition. The DC power flow approximation is a common approach for calculating load flow and detecting overloaded branches. This approximation

considers some assumption to achieve the linearization of power flow equations which results in reduction of computational burden. The accuracy of DCPF depends on these assumptions' validity in real network situation. These assumptions do not hold true for AC power flow which may result in huge computational burden and convergence problem for large complex network. On the other hand, the accuracy of AC power flow solutions is much higher and we can access the voltage profile of the busses in the network. Due to these approximations DCPF underestimate the severity of cascading failure in large complex networks [5]. In our proposed cascading failure model, we have used ACPF to detect overloaded branches. However, to determine the mean flow in the branches Unscented Transformation (UT) method is used to avoid the disadvantages of ACPF [10], [11].

C. Unscented transformation

The unscented transformation (UT) method can overcome the limitations of linearization by providing a direct and definitive approach for transforming statistical information. UT method can provide higher accuracy with the same computational burden as linearization. The basic idea of UT method is that it is easier to estimate a probability distribution function than it is to estimate an arbitrary nonlinear function [12]. In UT method, the input points are selected in a way that they can maintain enough information to represent their probability distribution function.

The UT method is applicable to different uncertain problems with satisfactory result. This method calculates the statistics output random variables undergoing a set of nonlinear transformations. In our model, the inputs of the UT methods are the load demand and renewable generation. We have chosen the input points in a way so that we can determine the mean and covariance of the input variables. Then UT method can estimate the mean and covariance of the output random variables, in our case, line flow in the network [7]. Special focus should be given on the idea that, in the UT method, the sample points are not selected randomly. They are chosen in a specific way so that they have a predefined mean and covariance. This statistical information propagates through some nonlinear function and ultimately results in an accurate estimation of statistics of the output variable.

D. Tripping mechanism and relay model

In the proposed relay model, we have used the mean and covariance of the branch flow derived by the UT method. This statistical information is the main component of the CF simulation relay model. Here, power flow of each branch is assumed to be Gaussian to determine the normalized overload distance and in result, the overloading probability of each branch [3]. Then, we can calculate the mean overload time ($\overline{\tau_l}$) of branch l using the normalized overload distance (z_l) and overloading probability (ρ_l).

$$\overline{\tau_l} = \frac{2\pi\rho_l e^{\frac{z_l^2}{2}}}{BW_l} \quad (2)$$

Where, BW_l is the equivalent bandwidth of the flow process for the lth line [13].

This relay model introduces the uncertainties injected from RE sources and loads to the line flows. The time-inverse relay algorithm is in motion when the line becomes overloaded and the time to trip (t_{tr}) is inversely proportional to the line overloading value. This value is determined based on the thermal stability of the transmission lines [14]. This t_{tr} value is compared to the mean overload time, $\overline{\tau_l^u}$, if it is larger the trip timer is set to zero, otherwise, the trip timer is set to the relay time to trip. This tripping mechanism enables us to model the stochastic process of CF and identify the most probable path for its propagation.

3. MODIFIED OPF ALGORITHM

During cascading failure process, branches of the power grid may trip and become disconnected. The grid network changes after every line trip. To restore the balance in the system some modification may be required. In [7], author proposed a power balance algorithm to restore the balance. In this algorithm, conventional OPF is used which could not accurately evaluate the impacts of RE penetration into the grid during CF. As CF is a very fast evolving process, conventional OPF would not properly reflect the transition path of CF process after every line tripped. Here, to mimic the most likely evaluation path of CF process in each step of the simulation process, we proposed a modified OPF algorithm which can restore the balance of the grid within its capacity limit. In this algorithm, we have proposed least square adjustment of the parameters of power grid within their valid capacity limit. The solution of this optimization problem restores the balance of the grid network within the least possible adjustment of generation and load controls in the grid and in result, accurately evaluates the impacts of RE penetration during CF process by representing the most likely transition path of CF process after every line trip.

A. Objective function

The objective function of this modified OPF is the least square adjustment of generation and load controls after every line tripping occurs. In this optimization problem, we try to minimize the differences of generation and load control parameters from their initial value. As oppose to the conventional OPF, it only focusses on real and reactive generation, real and reactive load demand and bus voltage magnitude. In CF process, every line tripping result in change of the existing network and the adjustment of generation and load is necessary. The modified OPF serve this purpose keeping the system as close to the initial condition.

$$\min_{P_G,P_D,Q_G,Q_D,V} \boldsymbol{W}[\ \|\Delta P_G\|_2^2\ \|\Delta P_D\|_2^2\ \|\Delta Q_G\|_2^2\ \|\Delta Q_D\|_2^2\ \|\Delta V\|_2^2\ ]^{\boldsymbol{T}} \quad (3)$$

Here, $\Delta P_G, \Delta Q_G, \Delta P_D, \Delta Q_D$ and ΔV represent the real power generation adjustment, reactive power generation adjustment, real load adjustment, reactive load adjustment and the voltage magnitude adjustment respectively for each bus before and after line tripping.

And, $\boldsymbol{W}$ represents coefficients or the penalty factors for the respective terms in optimization problem.

Here, $\boldsymbol{W} = [\ w_1\ w_2\ w_3\ w_4\ w_5]$. The values of these coefficients determine the gravity of their respective terms in the overall minimization problem. For example, in a specific case when minimizing load shedding is of top importance, one can put extra effort in minimize it by setting higher value of w_2 from other coefficients, which may result into other parameters shift away from initial value. In other cases, it could be of more importance to keep the differences of generation dispatch as less as possible. In that case, coefficient w_1 will be set to a higher value to minimize the difference of generation dispatch which may lead to higher amount of load shedding. Thus, the values of the coefficients can be chosen in a way to serve specific purpose in specific cases. Here, in our simulation, all the coefficient values are set to 1 to give equal importance to every term in the modified OPF algorithm.

B. Constraints

The modified OPF is a constrained optimization problem which has two types of constraints.

Equality Constraint: The active and reactive power balance in each bus, namely the AC power flow equation is the equality constraint set for this modified OPF.

$$f(V_m, \theta, S_{G,} S_D, Y) = 0 \quad (4)$$

Here V_m represents bus voltage magnitudes, θ represents bus voltage angles, S_G is the complex generation power, S_D is the complex load and Y is the admittance matrix for the grid after line tripping occurs.

Inequality constraint: The operating limits of the components of power grid control the steady state operation of the network. These operating limits are the inequality constraints set for this modified OPF.

$$X_{min} \leq X \leq X_{max} \quad (5)$$

Here X represents real and reactive generation, real and reactive load, bus voltage magnitude and bus voltage angle, X_{max} is the upper boundary and X_{min} is the lower boundary of the parameters. These are the inequality constrains of the modified OPF.

C. Algorithm

In CF simulation process, after every line tripping this modified OPF restores the balance of the network using least square adjustment of the generation and load controls

Figure 1 shows the flowchart of the modified OPF. This modified OPF model represents a nonlinear constrained optimization problem. This modified OPF takes the transformed admittance matrix after tripping and power flow solution of the network before line tripping as inputs. The parameters are initialized as the power flow solution of the network before line tripping. The inequality constraints are set as the operating limits of the generation and load controls and the equality constraints are the real and reactive power balance equations for each bus.

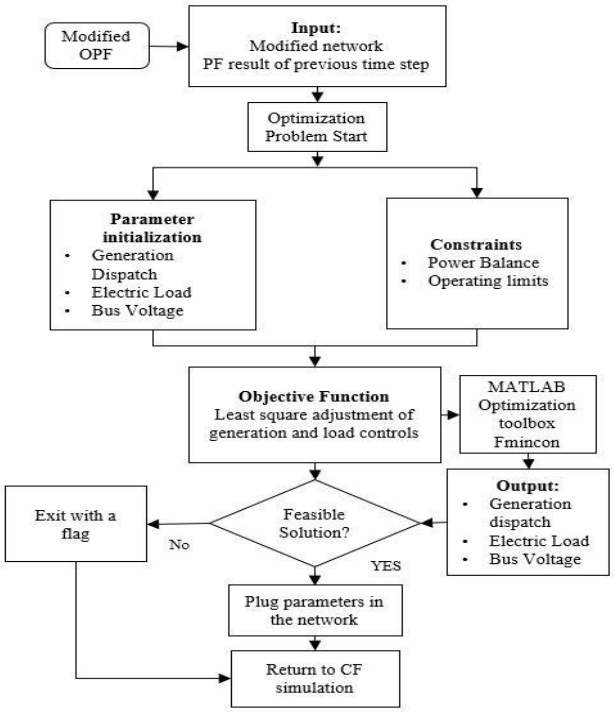


Fig 1. Flowchart of the modified OPF

For a specific network we have chosen a few different scenarios where several generators were selected to replace them with wind generators. For every scenario, we have chosen different conventional generators for replacement. According to the load data, wind data and scenarios, we performed an initial optimal power flow to determine the generation dispatch for different RE penetration. Then we introduced n-2 contingency into the system to initiate cascading failure. After every line tripping, the modified OPF restored the power balance of the network with least square adjustments of the parameters within their boundary. In every time step, we performed power flow analysis to determine the overloaded lines. For every overloaded line, we have used UT method to find out mean overloading time and specific lines have been tripped when the trip timer hit zero. During cascading process, we have shut down all isolated buses and islands without generation. After the simulation, load shedding amount and number of tripped lines have been stored. For every scenario, we have used several different n-2 contingencies to initiate cascading process and took the average of load shedding and trip count to measure the severity of the cascading process. For every scenario, we have repeated the process for different level of RE penetration to see the impact of RE penetration level on the severity of cascading process.

4. SIMULATION RESULTS AND DISCUSSION

We have simulated the impacts of increasing renewable generation integration on grid vulnerability to cascading overload failures in two IEEE standard cases (IEEE 118 and IEEE 300 bus system). In both cases, we have selected a few different scenarios and several different initial n-2 contingencies to initiate cascading process. We have used four hours of load data and wind generation data with 4 second resolution.

Scenarios: In a specific scenario, we have selected six different conventional generators to replace those with wind generators. The six conventional generators have been selected in a way so that they could contribute at least 30% of the total generation of the system which in result, represents up to 30% RE penetration in the system. Then these conventional generators are gradually replaced by wind generators to see the effect of increasing RE penetration in the grid. In our simulation, we have chosen a limited number of scenarios in order to reduce the computational burden. The scenarios have been chosen in a way so that most of the conventional generators by turn could have been replaced by wind generators.

Contingency: To initiate the CF simulation, we have manually tripped two branches (n-2 contingency) at the initial stage of the simulation. We have selected several different n-2 contingencies to initiate the CF simulation to see different cascading failure evaluation path for every scenario. In order to reduce the computational work load, we have selected limited number of initial n-2 contingencies. The contingencies have been selected from a batch of contingencies which mostly represents the critical lines of the system which leads to CF process.

IEEE 118 Bus System: The IEEE 118 bus test case has 54 committed generators, 99 loads and 186 branches. We have considered fifteen different scenarios where we replaced conventional energy with wind energy to see the impact of RE penetration in case of cascading failure.

To initiate cascading failure, we introduced 50 different N-2 contingencies in the simulation for every scenario to see its evolution process. To measure the severity of the cascading process we considered the load shedding amount and trip count

for every cases. For every scenario, we have tested our model for 0% to 30% renewable penetration. We have taken the mean of the total load shedding amount for 50 different initial n-2 contingencies.

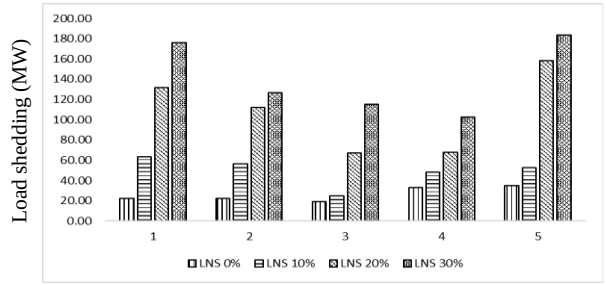
In scenario 1, generator 5, 10, 17, 21, 30 and 40 have been replaced by wind generator. We have simulated this scenario for 50 different n-2 contingencies. The average load shedding amount and average count of line tripping are the measuring tool to asses vulnerability to cascading failures.

TABLE I
VULNERABILITY IMPACTS EVALUATION (SCN 1)-IEEE118

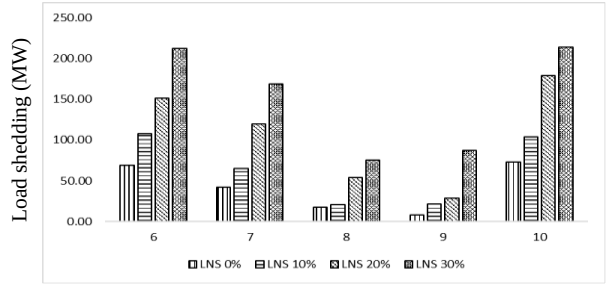
Avg Load Shedding (MW)				Avg number of lines tripped			
RE 0%	RE 10%	RE 20%	RE 30%	RE 0%	RE 10%	RE 20%	RE 30%
22.19	63.49	131.95	175.84	2.54	4.02	4.98	5.90

From Table I, we can see that for scenario 1, with increasing renewable penetration, the average load shedding amount increased from 22.19 MW to 175.84 MW. And the average trip count also increased from 2.54 to 5.90. The increasing renewable penetration increased uncertainty into the system which results in larger amount of load shedding which makes the network more vulnerable to cascading failure.

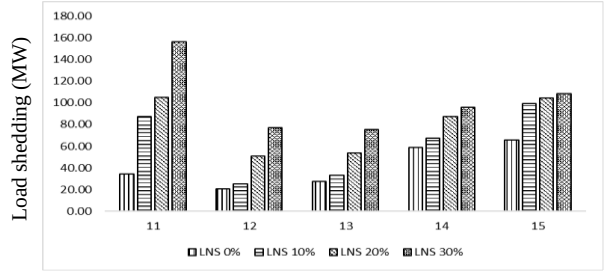
From figure 2, we can see the same trend for every scenario, which is the increasing trend of load shedding amount with increasing RE penetration.



Scenario (1-5)



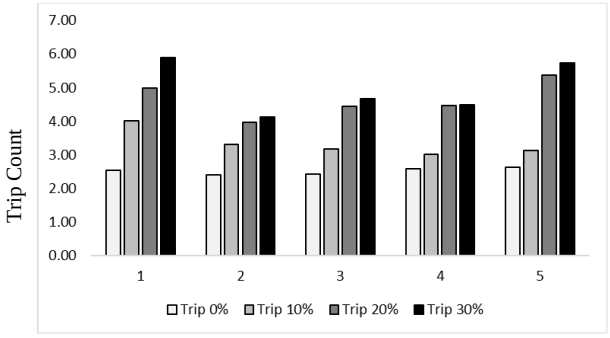
Scenario (6-10)



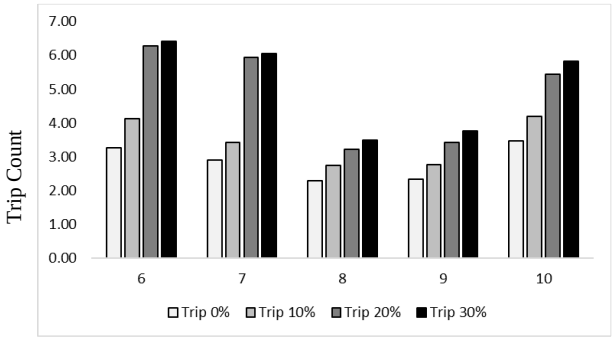
Scenario (11-15)

Fig. 2 Average load shedding amount for increasing RE penetration (IEEE 118 bus system)

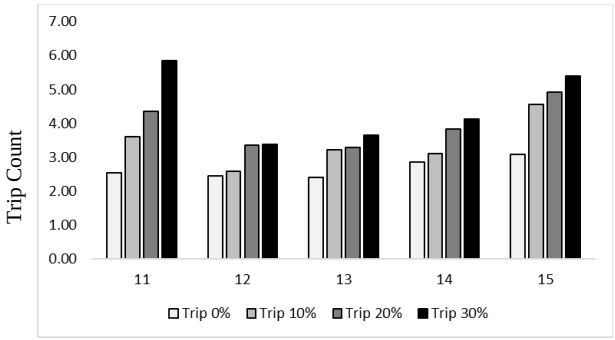
In figure 3, we have shown the average number of line trips for every scenario with 50 different n-2 contingencies. The line trip count also increased with the increasing RE penetration.



Scenario (1-5)



Scenario (6-10)



Scenario (11-15)

Fig. 3 Number of tripped lines for increasing RE penetration (IEEE 118 bus system)

For a specific scenario and for a specific contingency we can see the most probable cascading process evaluation path. In Table II, we can see that for scenario 1, when we initially tripped line 51 and 168 to initiate CF process, the trip count and the load shedding percentage increased with increasing RE penetration.

TABLE II
VULNERABILITY IMPACTS FOR A SPECIFIC CONTINGENCY-118 BUS

Scenario	N-2 Contingency	RE Penetration	Trip Count	LNS (%)
1	51, 168	0%	4	0.60
		10%	13	8.77
		20%	17	12.71
		30%	26	24.10

In figure 4, we can see the cascading failure evolution process for scenario 1 where branch 51 and 168 was initially tripped. We can see the escalation period in the initial stage and then after time step 2000 the system becomes stable.

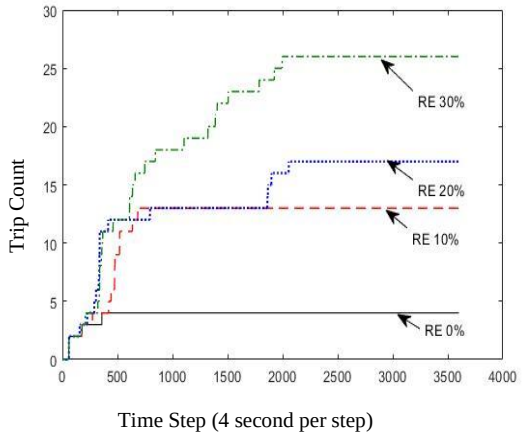


Fig. 4 Cascading failure evolution for a specific contingency

IEEE 300 Bus System: We have tested our algorithm on another IEEE test case, IEEE 300 bus system. This system contains 69 generators, 304 transmission lines and 195 loads, and its loading level is higher than 118 bus system. We have considered ten different scenarios for this test case where we replaced conventional energy with wind energy to see the impact in case of cascading failure. To initiate cascading failure, we introduced N-2 contingencies in the simulation. For every scenario, we have considered 10 different n-2 contingencies to initiate cascading failure and see its most probable evolution process. To measure the severity of the cascading process we have considered the load shedding amount and trip count for every case as measuring tool. For example, in scenario 2, generator 11, 14, 31, 48, 62 and 64 have been replaced by wind generator. We have simulated this scenario for 10 different n-2 contingencies. The average load shedding amount and average number of tripped lines are the measuring tool to asses vulnerability to cascading failures.

TABLE III
VULNERABILITY IMPACTS EVALUATION (SCN 2)-300 BUS

Avg Load Shedding (MW)				Avg number of lines tripped			
RE 0%	RE 10%	RE 20%	RE 30%	RE 0%	RE 10%	RE 20%	RE 30%
346.4	420.1	613.2	1181.9	4	4.67	6.22	8.11

From Table III, we can see that for scenario 2, with increasing renewable penetration, the average load shedding amount increased from 346.4 MW to 1181.9 MW. And the average trip count also increased from 4 to 8.11. The increasing renewable penetration increased uncertainty into the system which resulted into larger amount of load shedding and made the network more vulnerable to cascading failure.
From figure. 5 we can see the same trend for every scenario, which is the increasing trend of load shedding amount with increasing RE penetration.

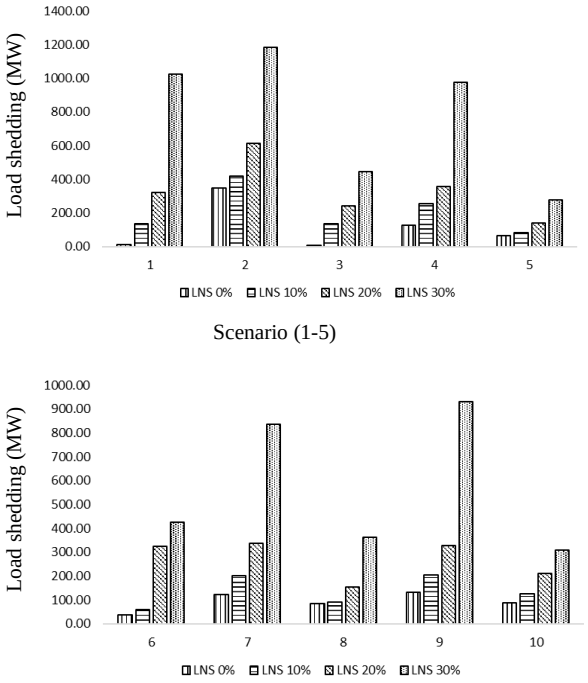


Fig. 5 Average load shedding amount for increasing RE penetration (IEEE 300 bus system)

In figure 6, we have shown the average number of line trips for every scenario with 10 different n-2 contingencies. The line trip count also increased with the increasing RE penetration.

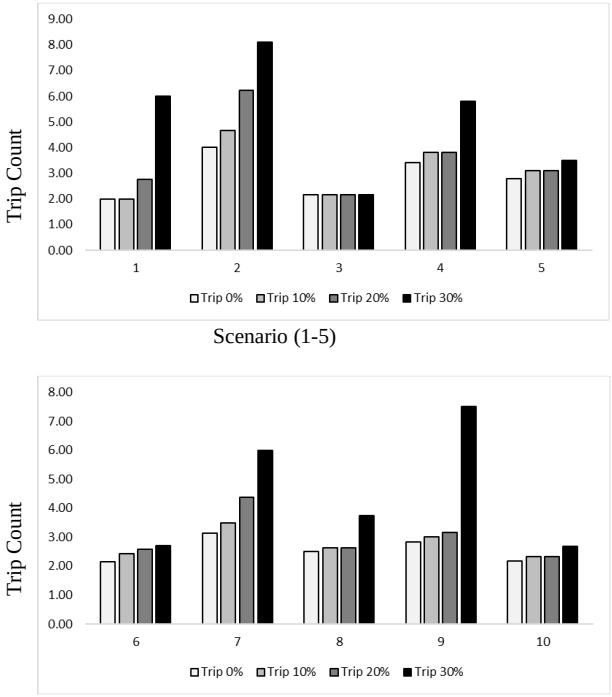


Fig. 6 Number of tripped lines for increasing RE penetration (IEEE 300 bus system)

Comparing the results of 118 bus system and 300 bus system, we can say that, in both cases with increasing penetration of RE, the amount of load shedding and the number of tripped lines increased. As measuring tool of vulnerability to cascading failure, the system becomes more vulnerable to cascading failure. Moreover, from figure 5, we can notice sudden rapid increase of load shedding amount for most of the scenarios in 300 bus system when RE penetration increases from 20% to 30%. As a matter of fact, on an average there is around 120% increase of load shedding when RE penetration increased from 20% to 30%.

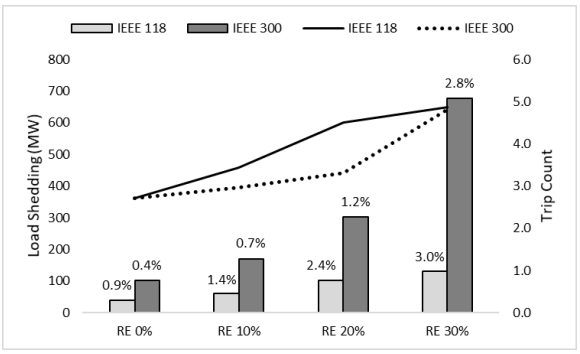


Fig. 7 Overall vulnerability analysis

From figure 7 we can see the overall vulnerability analysis for IEEE 118 bus system and IEEE 300 bus system averaging all the scenarios. We can see that for IEEE 118 bus system, load shedding reached 2.35% for 20% RE penetration. And for IEEE 300 bus system, there is a sudden increase in load shedding (2.8%) when RE penetration reached 30%. It is evident that when RE penetration reached a certain point, the system may enter a critical stage where it can deteriorate rapidly and preventive measures should be taken to mitigate the negative impacts of RE penetration. Hence, by incorporating the modified OPF power balance algorithm in simulation process, the negative impacts introduced by RE on the grid vulnerability have been accurately reflected.

5. CONCLUSION

In this paper, the impacts of renewable generation on grid vulnerability to cascading overload failures in terms of its penetration level was analyzed. A full ACPF model with UT method and modified OPF for power balance was used in the simulation process to accurately mimic the most likely evolution path of CF process and to make the study more accurate. Blackout size and number of tripped lines are used as measuring tools to asses vulnerability to CF. It is found that higher penetration from renewable energy leads to higher number of trip count and larger amount of load shedding. It has been also shown that after a certain level of RE penetration, some system may deteriorate rapidly and preventive measures should be taken if and when RE penetration level exceeds that limit. As a future extension of this study, it is of interest to incorporate mitigation tools to prevent or minimize the severity of cascading failure in case of higher penetration of renewables.

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Seasonal effect of aerobic exercise on lipid consumption

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ABSTRACT

Physical exercise is important for limiting fat mass and preventing obesity. Aerobic exercise after breakfast is effective in helping to maintain lipid consumption. Participants in this study included nine young Japanese women with body mass index (BMI) 19.0–23.5 who did not exercise regularly. Participants engaged in 30 min of aerobic exercise with loads equivalent to 45%, 50%, 55%, 60%, and 45% of maximum heart rate in sequential 6-min intervals during summer and winter. Respiratory metabolism and heart rate were measured using a bicycle ergometer. The amount of energy, lipids, and carbohydrates consumed was calculated using respiratory quotients. Energy and carbohydrate consumption and heart rate increased significantly compared with the resting control period, that increased linearly with increased exercise load. However, lipid consumption was not different across different exercise loads during both seasons. We calculated the difference of lipid consumption between winter and summer (Y). Our findings show that the relation between the difference in lipid consumption (Y) and BMI (X) was a significantly straight line ($Y = -23.6 X + 21.3$, $R2 = 0.628$). That is, participants with BMI >21.3 can more easily reduce body fat in summer, whereas those with BMI <21.3 can more easily reduce body fat in winter.

Keywords: Lipid consumption, Seasonal change, aerobic exercise, Respiratory metabolism, Young Japanese adult female.

1. INTRODUCTION

The main causes of illness-related death since World War II in Japan have changed from infectious diseases, such as tuberculosis or pneumonia, to geriatric diseases such as cancer, heart disease, and cerebrovascular disease. The onset and progression of geriatric diseases have been attributed to aging; however, it is clear that lifestyle is closely related [1]. In addition, because symptoms of these geriatric diseases have increased in children because of lifestyle factors [2], the term geriatric diseases were changed to lifestyle-related diseases in 1996 by the Ministry of Health, Labour and Welfare. One cause of lifestyle-related diseases is obesity, and it is said that approximately 90% of obesity cases are simple obesity. Obesity [3/4] is a contributing factor to geriatric diseases such as diabetes [5], high blood pressure [6], dyslipidemia [7], myocardial infarction [8/9], and cerebral infarction [10].

To prevent obesity and improve health, proper eating habits and regular exercise are important. In addition, it is necessary to keep body fat (BF) at an appropriate level. Excessive caloric intake [11] and lack of exercise contribute to reduced fitness and possible long-term illness. Vigorous exercise is considerably demanding for a person unaccustomed to such activity. According to Mersy et al. [12], aerobic exercise benefits health and Ootani [13] confirmed that this was by affecting lipoprotein metabolism in sedentary healthy young women. Thus, we used aerobic exercise in this study, measured with an ergometer, as a simple way to assess the work performed during exercise. This study aimed to identify easily achievable conditions in different seasons that could enhance BF metabolism during aerobic exercise by assigning moderate exercise in combination with normal eating habits [14-16].

2. METHODS

Participants and experimental loads for measuring respiratory metabolism

The physical constitution of participants and their exercise loads are shown in Table 1. Participants included nine young Japanese women in their 20s who were nonsmokers, had no history of systemic disease, and were not engaged in any physical training or dietary program. Respiratory metabolism was measured in the follicular phase of participants' menstrual cycle [17] during the summer and the winter.

Table 1 Participants' average physical constitution and exercise loads

Age (Year)	Height (m)	Weight (kg)	BMI ($kg \cdot m^{-2}$)
21.7 ± 1.0	1.6 ± 0.1	53.9 ± 5.8	21.2 ± 1.6
Exercise load (W)			
The 1st Step	The 2nd Step	The 3rd Step	The 4th Step
25.0 ± 3.5	41.1 ± 2.2	55.0 ± 3.5	68.9 ± 6.0

Experimental schedule

Participants awoke at 06:00 after 7 h of sleep, who consumed a prescribed breakfast [18/19] at 07:30. The young women then entered a climate-controlled room (ambient temperature 24.5 ± 0.3°C; relative humidity 50.3% ± 2.5%; luminance 827 ± 27 lx; and airflow rate 8.0 ± 0.1 cm/s) at 10:30, 1 h before the experiments. BF percentage, muscle mass, bone mass, and basal metabolism were measured using an electric bio-electrical impedance analysis scale (BC-520, TANITA, Tokyo, Japan). Body weight was measured using a balance (IPS-150 K, Shimazu, Kyoto, Japan). Participants were seated at rest for about 1 h while wearing a short-sleeved 100% cotton T-shirt and 100% polyester running pants. Respiratory metabolism levels were measured with a Vmax-229 (Sensormedics, CA, USA), using the

breath-by-breath method, for 40 min. Participants engaged in 30 min of aerobic exercise with loads equivalent to 45%, 50%, 55%, 60%, and 45% of maximum heart rate (HR) using the Karvonen equation [20] in sequential 6-min intervals (the second 45% interval served as a cool-down period). Respiratory metabolism and HR were measured during exercise and 5 min before and after, as a control.

Calculation of energy/carbohydrate/lipid consumption

BF metabolism was assessed by measuring respiratory metabolism before, during, and after aerobic exercise. The device used measured the amount of inhaled oxygen (L/min) and exhaled carbon dioxide (L/min) for every lungful of air. Energy consumption (EC) per 1 L of oxygen (kcal/O_2l) was calculated by obtaining the respiratory quotient (RQ) [21] using a Zunts–Schumberg–Lusk table [22], to analyze oxidation of the mixture of carbohydrate and fat. EC was calculated using the following equation.

$$EC = 1.22RQ + 3.83 \text{ (unit: kcal/}O_2\text{l)} \quad (1)$$

This was used to determine the EC ratio supplied from carbohydrate versus lipid metabolism. The carbohydrate ratio Z for EC was determined using the equation:

$$Z = 3.17RQ - 221.81 \quad (2)$$

The lipid ratio X was calculated as:

$$X = 100 - Z \quad (3)$$

Therefore, the amount of energy consumed (kcal/O_2 l) during respiration was divided into the relative EC (kcal/min) supplied by carbohydrate consumption (CC) or lipid consumption (LC) using oxygen intake (L/min). Next, carbohydrates and lipids metabolized during respiration were calculated using the relationship of 4.1 kcal per gram of carbohydrate and 9.3 kcal per gram of lipid [23].

Statistics

From respiration metabolism determined using the breath-by-breath method, the averages of EC, CC, LC, and HR were calculated every 10 s. EC, CC, LC, and HR measured before exercise at rest were compared with those from each exercise step using a paired t-test in consideration of individual differences. Significant findings between each exercise stage were confirmed in EC, CC, LC, and HR from stages 1 to 4. The correlation coefficients between LC and the following relative factors were calculated: BMI, basal body temperature/basal metabolism, sweat/insensible water loss, BF/visceral fat level, and muscle/bone mass.

3. RESULTS AND DISCUSSION

RQs of each participant (denoted A to I) in summer and winter are shown in Figure 1. The left- and righthand figures represent summer and winter, respectively. RQs changed by season among individual participants. LC was calculated using these RQs and the abovementioned equations.

Figure 2 shows a typical example of a participant's energy, carbohydrate, and lipid consumptions and HR in summer. EC, CC, LC, and HR were observed to increase with exercise load level. However, LC did not change with increasing exercise loads. The results for EC, CC, LC, and HR of all participants were then calculated at each exercise load, shown in Figure 3. Significant differences for each exercise step between summer and winter, and during summer and winter, are shown in Table 2.

There were no significant differences between summer and winter for EC, CC, LC, and HR among all participants at each exercise load. That is, EC, CC, LC, and HR did not change under the same exercise conditions in the different seasons. However, the combinations between each step in EC, CC, and HR in summer and winter showed significant differences. EC, CC, and HR increased significantly with increasing exercise load, according to each season. LC showed no significant findings, except for some combinations: BC (before resting control) 1/4/1' pairs in summer, BC-1/2/3/4/1'pairs, 1–3 pair in winter). That is, LC did not increase with exercise load level. As we described previously [20], only a light exercise load of around 25 W is needed to reduce BF. The findings regarding LC are discussed below. LC in summer and winter is shown in Figure 4.

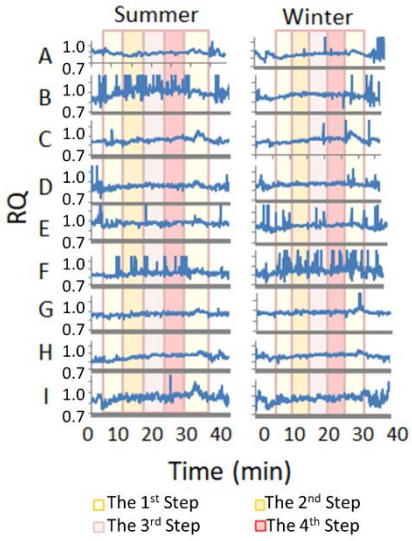

Figure 1 Respiratory quotient (RQ) of each participant

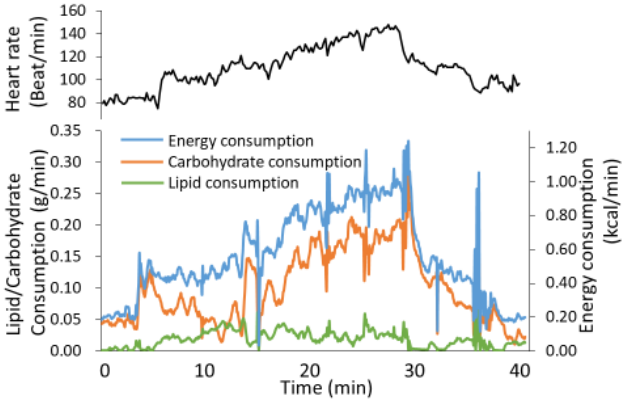

Figure 2 Typical example of EC, CC, LC, and HR in summer (Participant A)

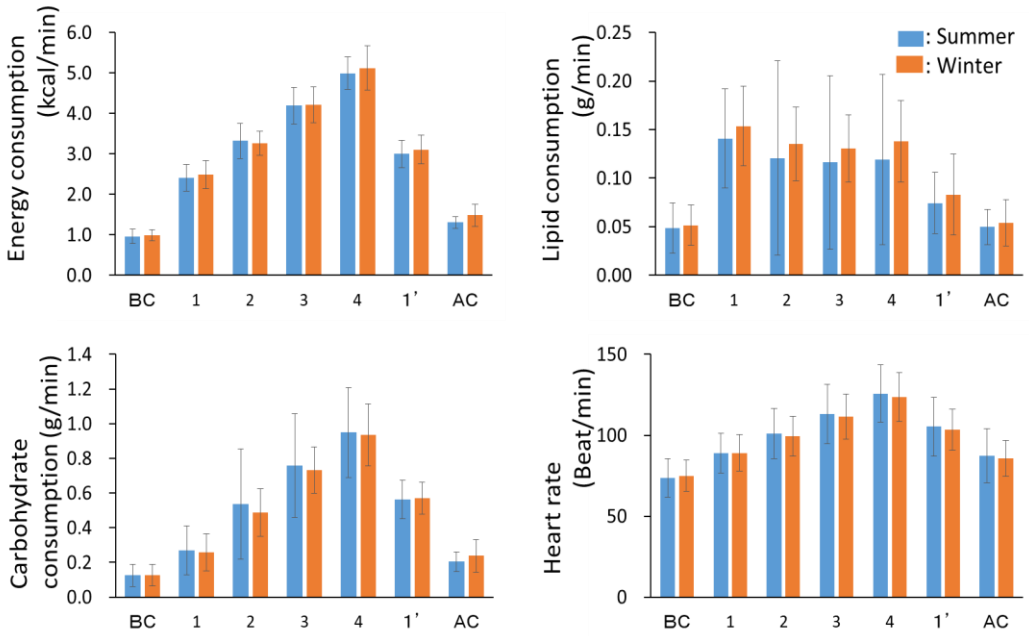

Figure 3 Difference between summer and winter for EC, CC, LC, and HR in all participants at each exercise load

Table 2 P values of EC, CC, LC, HR (two-sided test) and the judgement between each exercise step in summer and winter

Pair	EC		CC		LC		HR	
Summer–Winter	Pvalue	Judge	Pvalue	Judge	Pvalue	Judge	Pvalue	Judge
BC–BC	0.469	–	0.957	–	0.808	–	0.695	–
1–1	0.273	–	0.824	–	0.556	–	0.976	–
2–2	0.617	–	0.633	–	0.664	–	0.710	–
3–3	0.901	–	0.782	–	0.631	–	0.700	–
4–4	0.352	–	0.907	–	0.606	–	0.612	–
1'–1'	0.176	–	0.816	–	0.606	–	0.668	–
AC–AC	0.054	–	0.298	–	0.697	–	0.673	–
Summer								
BC–1	0.000	***	0.004	***	0.000	***	0.000	***
BC–2	0.000	***	0.005	***	0.074	–	0.000	***
BC–3	0.000	***	0.000	***	0.068	–	0.000	***
BC–4	0.000	***	0.000	***	0.049	*	0.000	***
BC–1'	0.000	***	0.000	***	0.050	*	0.000	***
BC–AC	0.000	***	0.003	***	0.879	–	0.001	***
1–2	0.000	***	0.010	***	0.494	–	0.000	***
1–3	0.000	***	0.000	***	0.352	–	0.000	***
1–4	0.000	***	0.000	***	0.375	–	0.000	***
2–3	0.000	***	0.000	***	0.587	–	0.000	***
2–4	0.000	***	0.000	***	0.892	–	0.000	***
3–4	0.000	***	0.001	***	0.765	–	0.000	***
Winter								
BC–1	0.000	***	0.000	***	0.000	***	0.000	***
BC–2	0.000	***	0.000	***	0.000	***	0.000	***
BC–3	0.000	***	0.000	***	0.000	***	0.000	***
BC–4	0.000	***	0.000	***	0.000	***	0.000	***
BC–1'	0.000	***	0.000	***	0.030	*	0.000	***
BC–AC	0.000	***	0.003	***	0.696	–	0.000	***
1–2	0.000	***	0.000	***	0.238	–	0.000	***
1–3	0.000	***	0.000	***	0.046	*	0.000	***
1–4	0.000	***	0.000	***	0.209	–	0.000	***
2–3	0.000	***	0.000	***	0.505	–	0.000	***
2–4	0.000	***	0.000	***	0.841	–	0.000	***
3–4	0.000	***	0.000	***	0.420	–	0.000	***

LC showed no significant difference between summer and winter; however, there were significant differences between control rest periods and during exercise (paired t-test). That is, LC was significantly increased two to three times by exercise. Correlation coefficients were calculated between LC and some factors (BMI, basal body temperature/basal metabolism, sweat/insensible water loss, BF/visceral fat level, and muscle/bone mass), shown for summer, winter, and the value for summer LC deducted from winter LC. No significant correlations were obtained between summer LC and other factors except BF-exercise period. In winter, the relationship between LC and BMI/BF/visceral fat level showed significant correlations between the control and exercise periods. Furthermore, the value of summer LC deducted from winter LC showed significant correlations between BMI/BF/visceral fat level. The relationship between BMI and LCs in control and exercise conditions are plotted in Figure 5. The left- and

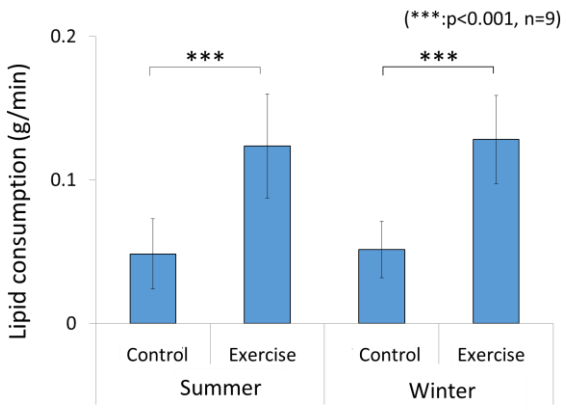

Figure 4 Lipid consumption in summer and winter

Table 3 Correlation coefficients between lipid consumption and its relative factors

Lipid consumption (g/min)	BMI ($kg \cdot m^{-2}$)	Basal body temp. (℃)	Sweat (g)	Body fat (%)
Summer				
Control	0.347	−0.267	−0.006	0.186
Exercise	0.477	−0.231	−0.259	0.677 *
Winter				
Control	−0.874 **	0.396	0.133	−0.886 **
Exercise	−0.715 *	0.378	0.024	−0.699 *
Difference between Winter and Summer				
Control	−0.749 *	0.419	0.081	−0.641
Exercise	−0.788 *	0.401	0.203	−0.923 ***

Lipid consumption (g/min)	Bone (%)	Basal metabolism	Visceral fat level	Muscle (%)
Summer				
Control	0.513	0.541	0.387	0.519
Exercise	−0.196	−0.073	0.531	−0.241
Winter				
Control	0.051	−0.203	−0.861 **	0.069
Exercise	−0.122	−0.310	−0.752 *	−0.089
Difference between Winter and Summer				
Control	−0.339	−0.504	−0.771 *	−0.333
Exercise	0.067	−0.138	−0.851 **	0.120

righthand figures show control and exercise conditions, respectively. Significant straight-line relationships were obtained BMI (Y1: control condition, Y2: exercise condition) and winter − summer LC (Xa) using the following equations:

$$Y1 = -34.3(Xa) + 21.3 \ (R2 = 0.627) \quad (4)$$

$$Y2 = -23.6(Xa) + 21.3 \ (R2 = 0.628) \quad (5)$$

We revealed that participants with BMI >21.3 could more easily raise their LC in summer. Conversely, participants with BMI <21.3 could more easily increase LC in winter. LC was affected by BMI, and changes in the seasons. In this way, we recognized that burning BF effectively is related to BMI.

4. CONCLUSIONS

Aerobic exercise after breakfast is effective in helping to maintain lipid consumption. Participants in this study included nine young Japanese women with body mass index (BMI) 19.0–23.5 who did not exercise regularly. Participants engaged in 30 min of aerobic exercise with loads equivalent to 45%, 50%, 55%, 60%, and 45% of maximum heart rate in sequential 6-min intervals during summer and winter.

Respiratory metabolism and heart rate were measured using a bicycle ergometer. The amount of energy, lipids, and carbohydrates consumed was calculated using respiratory quotients. Energy and carbohydrate consumption and heart rate increased significantly compared with the resting control period, that increased linearly with increased exercise load. However, lipid consumption was not different across different exercise loads during both seasons. We calculated the difference of lipid consumption between winter and summer. Our findings show that the relation between the difference in lipid consumption (Y) and BMI (X) was a significantly straight line (Y = −23.6 X +21.3, R2 = 0.628). That is, participants with BMI >21.3 can more easily reduce body fat in summer, whereas those with BMI <21.3 can more easily reduce body fat in winter. According to BMI, it was revealed lipid consumption was affected by a season.

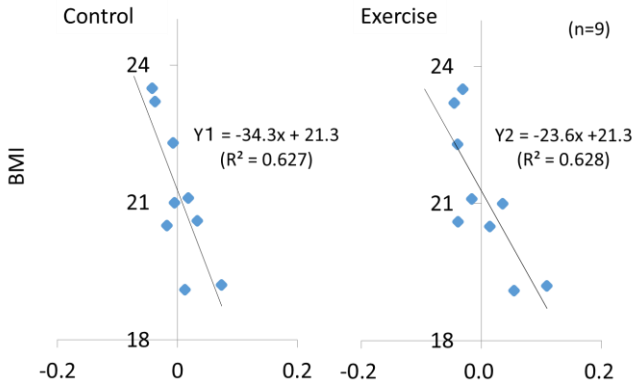

Figure 5 Relationship between BMI and value of summer lipid consumption (LC) deducted from winter LC

5. ACKNOWLEDGEMENT

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Abbreviations

BF: body fat
BMI: body mass index
CC: carbohydrate consumption
EC: energy consumption
HR: heart rate per minute
LC: lipid consumption
RQ: respiratory quotient
X: lipid rate for energy consumption
Xa: winter – summer lipid consumption
Y1: BMI with control condition
Y2: BMI with exercise condition
Z: carbohydrate ratio for energy consumption

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A systematic literature review protocol for evaluating the Strengths, Weaknesses, Opportunities and Threats (SWOT) of EHR and PDS

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ABSTRACT

The use of e-health applications has increased globally with advances in information and communications technology (ICT). These applications are effective for improved healthcare co-ordination and outcomes; however, contextual differences may exist in the Strengths, Weaknesses, Opportunities and Threats (SWOT) across various economies given differences in available resources for e-health implementation. This protocol details the steps for evaluating SWOT elements as they relate to implementation of the e-health applications, electronic health records (EHR) and pharmacy dispensing system (PDS), across various economies.
Keywords: SWOT, EHR, PDS, e-health

1. INTRODUCTION

The term, e-health, refers to the application of information and communications technology (ICT) in healthcare. It has found application in various health-related fields (care, surveillance, diagnostics, literature, knowledge, education, and research [1]. The concept of e-health has grown with wider use of the internet to become a convergence of ICT applications in medical informatics, public health, and business. This growth is not limited to technical development but has also become a way of thinking for healthcare improvement from local to global scenes [2].

The development of various e-health tools has increased over the years, accelerated by buy-in from global health institutions; for instance, the World Health Organization has urged member states to draw up sustainable strategies for e-health implementation as a tool for advancement towards universal health coverage [1,3]. Two of the major e-health applications that are effective in streamlining records in different environments are electronic health records (EHR), also known as electronic medical records (EMR), and the pharmacy dispensing system (PDS). These have the potential to transform healthcare, with key roles in record keeping and drug dispensing to improve patient care, and their implementation can vary across different environments. The benefits and challenges associated with consideration of context for EHR and PDS have been documented;[4–7]. however, very little exists on how contextual differences across economies may influence the Strengths, Weaknesses, Opportunities for innovation, and Threats (SWOT) of EHR and PDS implementation.

The SWOT method has found application in various fields though the origin of SWOT is still under debate [8–10]. As an organizational evaluation method, SWOT generally highlights the various elements of a system; the potential that can be harnessed for growth as well as the threats the business needs to be aware of to operate securely and efficiently [11]. In this protocol, the research focus will be on the SWOT elements (and not on the SWOT method) which will be used to evaluate EHR and PDS. The purpose of this protocol is to outline the steps for conducting a systematic literature review on the strengths, weaknesses, opportunities, and threats (SWOT elements) of EHR and PDS across low-, middle- and high-income countries.

2. METHODS

This protocol has been developed for the conduct of a systematic literature review as detailed by Xiao and Watson [12]. While various approaches to literature review exist, it is important to note that most reviews are conducted following eight simple step-by-step processes], hence the protocol is according to these steps:

1. Formulation of the review question
2. Development and validation of the review protocol
3. Literature search and extraction of records
4. Records screening
5. Quality assessment/critical appraisal
6. Data extraction
7. Data synthesis
8. Report/communication of findings

In addition, the researchers found it useful to incorporate additional element of evidence-based research in the formulation of the review question.

2.1. Formulation of the Review Question

A key part of any systematic literature review is the development and specification of the research question(s) [13]. This is essential as it provides a framework upon which the conduct of the entire systematic literature review rests.

Once the research question(s) is/are formulated, the inclusion and exclusion criteria for the research can be developed by focusing on the concepts identified in the research question(s). Having a detailed and well-defined research question provides clear guidance to facilitate the drawing of well-informed conclusions.

For evidence-based research, the usual framework for generating the research question is that developed by Richardson et al. as referenced in Davies: the PICO framework [14,15]. PICO is an acronym used to generate research questions in evidence-based research, where **P** stands for **P**atient**, P**opulation or **P**roblem; **I**

for **I**ntervention; **C** for **C**omparison; and **O** for **O**utcome. The systematic review for which this protocol is being developed did not fit the PICO elements. Following discussions by the researchers, an alternative research question generation framework, CLIP [16], with criteria and applicable descriptions, is presented (Table 1).

Table 1: CLIP framework for generation of research question

Criterion	**Descriptions**
Client	Health care organisations (clients of EHR and PDS services, e.g., government and private corporations)
Location	Various economies (low-, middle- and high-income countries)
Impact (or outcome)	To explore the differences in SWOT elements associated with EHR and PDS implementation
Professionals	ICT organisations involved in developing and maintaining e-health services

Research question: How does the implementation of EHR and PDS differ across various economies in terms of associated strengths, weaknesses, opportunities, and threats?

2.2 Development and Validation of the Review Protocol

A systematic review protocol is developed to guide the researchers in the conduct of the review. As such, planning and documenting the review method(s) (in a protocol) prior to the review helps to reduce bias, increase reliability, and improve the quality of the systematic review.

This protocol is developed for the purpose of conducting systematic literature review on SWOT elements of EHR and PDS. The research aims to explore how the SWOT elements related to EHR and/or PDS implementation differ across various economies.

2.2.1 Literature search and extraction of records: For this research, identification of relevant studies will be conducted following keywords search across four databases: EBSCO, PubMed, Scopus, and Web of Science.

The following keywords will be utilized (revised for each database as applicable:

(Streng* OR Benefit OR Advantage ... OR Challeng* OR Disadvantage OR Downside OR Limit* OR Vulnerab* ... OR Opportunit* … OR Threat OR Risk OR Danger OR "SWOT") AND (("EHR") OR (EMR) OR ("PDS"))

The researchers will also identify additional related keyword terms to expand the reach and extent of the search. The titles and abstracts of identified studies will be reviewed to exclude articles that will not contribute to answering the research question. Following preliminary assessment of the titles and abstracts, full texts of selected articles will be screened for relevance and applicability to the research topic. The reference lists of studies which meet the inclusion criteria will also be searched for relevant publications.

The reference management software, Mendeley© (Mendeley Limited), will be used to save and store articles retrieved from literature searches. Articles saved in Mendeley would be transferred to ATLAS.ti®, NVivo® or other suitable software application that will assist in data synthesis. The PRISMA flow diagram will be used to depict the processes followed to arrive at the selected literature for inclusion in the research.

2.2.2 Records screening: The records extracted will be screened by two independent reviewers. Full text versions of the relevant records will be evaluated using the inclusion criteria; peer-reviewed journal publications available in the English language, and articles published within the last 10 years. Studies will be excluded if they focus on any other aspect of e-health apart from EHR and PDS, and if they only report on the intention to implement EHR or PDS. Disagreements between the reviewers will be resolved by discussion or appointment of a third independent reviewer.

2.2.3 Quality assessment: Quality assessment is a method of further screening for inclusion or exclusion of studies as appropriate. It enables the reviewer to clearly justify the quality of articles selected for inclusion in the systematic review [17]. To assure quality, the reviewers will critically appraise the selected literature for relevance to the research. Relevant studies will be those grounded in the field of EHR and/or PDS implementation and their related SWOT elements.

2.2.4 Data extraction: The aim of data extraction in a systematic review is to collect information needed to answer the review question(s) [13]. The design of a data extraction form in the review protocol helps reduce error and bias in the review process. The data extraction form for the systematic literature review contains the categories that will assist in the classification of each selected article (Table 2).

Table 2: Data extraction form

General information of the study	
Title	
Author(s)	
Publication year	
Study country/countries	
Citation (database)	
Study characteristics	
Purpose of the study	
Study method(s)	
Data collection method(s)	
Classification schemes	
Strengths:	
Weaknesses:	
Opportunities:	
Threats:	
Study findings	
Any additional information	

2.2.5 Data analysis and synthesis: Data synthesis is performed to draw inferences from the overall analysis of available evidence and can be integrative or aggregative. In integrative synthesis, the key concepts for data summaries are assumed to be well defined prior to synthesis whereas this is not the case for aggregative synthesis [18]. Integrative synthesis is the primary data synthesis method that will be utilized for the proposed review. The concepts of *Strengths*, *Weaknesses*, *Opportunities*, and *Threats* are well defined and form the categories for summarizing data extracted from the selected articles.

3. DISCUSSION

This manuscript describes a systematic literature review protocol for exploring contextual differences in the implementation of EHR and PDS across various economies. The strength lies in the application of a reproducible and transparent process for review of the literature. Publication of this protocol also minimizes the risk of bias in the review. Limitations include the restriction to peer-reviewed journal publications, and the fact that the review will only identify documented, and not necessarily, all contextual details.

The proposed systematic literature review will present findings on differences in SWOT elements related to implementation of EHR and PDS across different economies. It will also explore the contextual details that lead to such differences and in so doing, highlight the SWOT elements that need more attention across different environments.

4. AMENDMENTS

In the event that any aspect of this protocol requires amendment, the reviewers will document the date, the amendment required and rationale for the said amendment.

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Regional Pet Database: an Information System to Guarantee Pet-Population Control

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ABSTRACT

An epidemiological approach to the study of pet population is needed to ensure the application of effective public health policies, addressed in particular to the management of monitoring plans and veterinary controls in the urban environments, where the human-pet interaction is closer. Since 1993, a regional database for pet-animals (dogs, cats and ferrets), called BAC, has been implemented in North-Eastern Italy (Veneto Region), involving both private and public Veterinary Services of the Local Public Health Units (LPHUs). BAC has been developed using the Oracle RDBMS platform with two applications ANACANI and ACWEB.
The information system includes data about owners and/or keepers, animals, their movements and events like vaccinations, neutering and dogs aggressions/bites. Data are processed and organized in reports useful to plan stray dog and cat population control strategies. Moreover, public Veterinary Services can use the system to monitor some social phenomena like owned-dog aggression and bites or pet abandon.

Keywords: pet, information system, population control, public veterinary services, Northeastern Italy.

1. INTRODUCTION

In Italy, as well as in other European Countries, about half of all households own a pet and especially dogs and cats - ownership is quickly increasing in urban environments[1]. Pet food industry estimated 77.400.000 cats and 68.500.000 dogs currently living in the European Union[2]. In 2019, according to the same source, Italian dog population was the sixth largest on following Russia, Germany, UK, France and Poland[3] with a human-dog ratio of 5[4]. The increasing trend in pet dog living in close contact with humans raises some safety issues for society and this is mainly due to bad dog management of some breeds, which have marked personality traits linked to aggressive behavior towards other dogs, unfamiliar people or household members[5]. Moreover, the abandonment of dogs and cats, as well as poor social tolerance towards cat colonies and stray animals, are very common in some urban context[6]. An epidemiological approach is needed to guarantee good management of pet populations and effective prevention policies to ensure people safety and animal welfare[7]. The estimate of canine and feline population size and the knowledge of its demographic structure are the base to set up surveillance plans and organize effective public health actions to control zoonosis and to prevent dog bites and aggressions[8]. The registration of owned dogs is therefore a necessity motivated by important social reasons. Identification and registration of dogs are necessary not only to control the canine population of an area but also to hold dog owners responsible and to take action in case of theft, loss or to contrast the phenomenon of neglected dogs.

Furthermore, pets can be useful sentinels of environmental contamination and comparative medicine can find in dogs registration and identification database an important epidemiological studies data source[9]. Since 1993, a Regional Database (RDB) for pets, called "BAC", has been implemented in North-Eastern Italy (Veneto Region). It involves Veterinary Surgeons, the Veterinary Services of the Local Health Units, the Regional Veterinary Service and Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe). All these users have access to the RDB and can visualize or manage data, according to their institutional role.

BAC was built to manage data about pet (e.g. dogs, cats and ferrets) and their owners or keepers. Both the Italian legislation framework (Law N. 281/1991; O.M. 6/08/2013)[10-11] and the European Regulations on pet non-commercial movements (Reg. EU 576/2013 and Reg. EU 577/2013)[12-13] pushed towards the development of tracing strategies mainly for dogs. They aim to reduce the number of stray dogs and cats and to identify dangerous dogs and their owners/keepers, to put in place preventive actions. In Italy, since 1991, dogs are required to be identified through a tattoo and, since January 2005, through the implantation of an electronic transponder, which is now the only dog identification system accepted by law.

2. THE REGIONAL DATA BASE FOR PET ANIMALS

BAC has been developed using the Oracle RDBMS platform. A specific webserver works with the latter, allowing interaction and processing of the data flow coming from the ACWEB and ANACANI applications. The first one is completely web-based while the second uses client-server technology and both have been developed using Embarcadero Delphi programming language. The web server used is Apache (Apache © Software Foundation), which manages applications requests in real-time and allows interaction with the web services. The communications technologies in use comprise XML and HTTP / SOAP standards.

Our information system includes:

a) a complete dataset about the animal (e.g. date of birth, breed, sex, size, coat color, transponder number, implantation site, implantation date, pet-passport code, address where the animal is housed);
b) personal data of the owner and the keeper (if existing);
c) tracing of animal movements (loss, death, theft, entrance into a shelter, adoption, owner return etc.) ;
d) the register of vaccinations, neutering;
e) the register of aggression and bite events with a risk classification of dogs, provided by the Local Veterinary Services.

Authorized veterinary surgeons possess their account to use the web-based tool ACWEB. They can register transponder implantation directly into the database with all the information related to the animal owner. Furthermore, they can record some kind of events (e.g. vaccinations, dog death). The Veterinary Services of the LPHUs use ANACANI application to manage data about dog population, cat colonies and shelter dogs. They register all changes in dog status (e.g. ownership, dog-housing place, dog loss, dog recovery and owner return). They record data about neutering campaigns addressed to stray cats and not-identified free-ranging dogs.

The Veterinary Services can also download specific reports about pet population, its distribution, the number of animals housed in each shelter or kennel and movements of animals from and to the territory of Veneto Region.

A special section of the ANACANI application is designed to allow registration and classification of dog aggressions and bites events in the framework of the Italian legislation[10-11]. The classification of dog aggressions and bites events allow us to have a picture of this phenomenon, investigating its features and to take some preventive actions towards animals and owners involved in these dangerous episodes.

All the institutional subjects cited above can constantly update data, so allowing the dematerialization, reducing costs and enhancing data availability.

3. DISCUSSION

Our information system represents an essential tool to control dogs and cats population at the regional level and allows a complete tracing of pet movements.

This reduces pet abandon and makes easier handing back a lost animal. The registrations of the vaccinations or the neutering represent an important source of data for the public veterinary authority that can support the decision-making process: this is essential in the management of public health issues and both epidemic or non-epidemic emergencies, related to pet animals. As an example, in 2008, after 20 years of absence, rabies re-emerged in the wild fox population of North-Eastern Italy. Besides measures adopted to fight the spread of the infection in wildlife, dog vaccination against rabies became mandatory in the risk area[14]. Our information system guaranteed an efficient organization and better control of the dog vaccination plan. Automated information processing allowed the supervision of vaccination operations, calculation of costs and monitoring of vaccination coverage of the dog population throughout the emergency.

An additional function provided by the system is the registration of dog aggressions and bites events in the framework of Italian law[11]. The phenomenon, monitored by the local Veterinary Services, allows a risk-based classification of dogs, and the implementation of specific educational programs addressed to their owner, to improve the dog-human relationship and owner responsibility. Moreover, epidemiological data analysis of the phenomenon can be used to verify the efficacy of the actions put in place.

The Regional BAC web-based tool guarantees the dematerialization of official data, enhancing their availability through a user-friendly interface and optimizing the communication in compliance with the European Data Protection Regulation[15].

4. CONCLUSION

BAC information system is useful to support the public veterinary services in dog and stray cat population control strategies. It provides epidemiological data to monitor pet population, particularly in the urban area, and some social phenomena linked to pet-animals that can affect public health, like dog aggressions or pet abandon. These data can help Public Authorities in their understanding of these social phenomena and support their decision-making processes about pet animal population management, including all preventive actions, like educational programs or communication campaigns addressed to the general public.

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13. Commission Implementing Regulation (EU) No 577/2013 of 28 June 2013 on the model identification documents for the non-commercial movement of dogs, cats and ferrets, the establishment of lists of territories and third countries and the format, layout and language requirements of the declarations attesting compliance with certain conditions provided for in Regulation (EU) No 576/2013 of the European Parliament and of the Council
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