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หัวข้อ
การศึกษาการแตกแขนงและการยึดติดของเซลล์ประสาทบนโลหะแก้ว
Branching and adhesion study of neurons on metallic glass

ทุนอุดหนุนการวิจัยจากเงินรายได้ ประเภทเงินอุดหนุนการวิจัยจากรัฐบาล
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สารบัญเรื่อง

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- นางสาวปฤณท์ธพร สงวนสุทธิกุล ภาควิชาคณิตศาสตร์ คณะวิทยาศาสตร์ มหาวิทยาลัยพระจอมเกล้าธนบุรี ระดับปริญญาเอก ชั้นปีที่ 1
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- นางสาวเสาวนีย์ จิตรศิลป์ฉายากุล ภาควิชาคณิตศาสตร์ คณะวิทยาศาสตร์ มหาวิทยาลัยพระจอมเกล้าธนบุรี ชั้นปีที่ 4

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- อาจารย์ นายแพทย์วีรภัทร โฆษิตานุฤทธิ์ แพทย์ต่อยอดสาขาโรคหัวใจ ชั้นปีที่ 2 ภาควิชาอายุรศาสตร์ คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

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บทคัดย่อภาษาไทย

จากการศึกษาก่อนหน้านี้ของกลุ่มวิจัยของเรา พบว่า วัสดุรองพื้นฟิล์มบางที่ทำมาจาก สารอสัณฐาน Au49Ag5.5Pd2.3Cu26.9Si16.3 เพิ่มการยึดติดและแบ่งตัวของเซลล์ Neuro2A ที่เลี้ยงแบบ in vitro ได้ดี ซึ่งเป็นข้อบ่งชี้ที่พบได้ไม่บ่อยนักบนวัสดุโลหะและโลหะผสม การศึกษาความสัมพันธ์ระหว่างการคงอยู่และการส่งกระแสไฟฟ้าของเซลล์ประสาทบนวัสดุทองโลหะผสมอาจเป็นการเปิดโอกาสใหม่ในการเชื่อมต่อเส้นประสาทที่สูญเสียไป การสร้างโครงข่ายระบบประสาทใหม่ และการชักนำให้เกิดการแตกแขนงของเส้นประสาท วัสดุทองโลหะแก้วมีข้อดีคือการขึ้นรูปของโลหะในรูปร่างและขนาดที่ต้องการ นำไปสู่การสร้างรูปแบบที่หลากหลายในโครงข่ายระบบประสาทได้ ในการศึกษานี้ วัสดุทองโลหะแก้ว วัสดุที่มีคุณลักษณะจำเพาะและมีความคาดหวังจะถูกนำมาขึ้นรูปแถบสามเหลี่ยมและสี่เหลี่ยมในระดับอนุภาคและนำไปเลี้ยงเซลล์ จะมีการใช้กระบวนการระเหยด้วยอุณหภูมิสูงในการยึดทองโลหะผสมเป็นวัสดุรองพื้น ความกว้างของแถบจะทดลองในขนาดที่หลากหลายเพื่อค้นหาขนาดที่ทำให้เกิดการยึดติดและการแบ่งตัวของเซลล์ที่ดีที่สุด ในการทดลองจะเก็บภาพรูปแบบอนุภาคด้วยกล้องจุลทรรศน์เรืองแสงชนิดหัวกลับ จากนั้นจะมีการทดสอบการยึดติดและการแบ่งตัวของเซลล์ เซลล์ Neuro2A จะถูกกระตุ้นให้แตกแขนงและยึดติดอยู่บนวัสดุทองโลหะผสมรูปแบบอนุภาค โดยมีการเปรียบเทียบการแตกแขนง ยึดติด และแบ่งตัวของเซลล์ที่อยู่บนวัสดุเทียบกับวัสดุแก้วทั่วไปและวัสดุแก้วที่เพิ่มสารโพลิเมอร์

บทคัดย่อภาษาอังกฤษ

In our previous *in vitro* Neuro2A cell culture study, amorphous Au49Ag5.5Pd2.3Cu26.9Si16.3 metal film substrate was found to promote neuron cell adhesion and proliferation. Such indication is not common for metal and alloys. The combination of neuron cell compatibility and electronic conductivity in the gold amorphous alloy has opened up a new opportunity in nerve repair, nerve reconstruction and neural induction. The ease of processibility in metallic glass can facilitate the patterning of the metal into desired shapes and sizes. In this study, gold-based metallic glass, a unique and promising material, will be micropatterned as triangular structure and rectangular stripes onto glass substrates and used in in-vitro neural cell culture. The process of thermal evaporation will be utilized to deposit the gold amorphous metal on to the substrates. Width of the pattern will be also varied for the triangular pattern to find the width that promotes Neuro2A cell adhesion and proliferation. The micropatterns will be characterized using inverted fluorescent microscope. Cell adhesion and proliferation tests will be conducted. The results will be obtained from the inverted fluorescent microscope. Neuro2A cells will be branched and adhered on micropatterned metallic glass substrate. The branching and adhesion of cells to these gold-based metallic glass micropatterns will be compared with positive control and unmodified glass substrates.

บทนำ (Introduction)

Tissue engineering is one of the most promising fields that have many clinical applications. Among all the complexities of the human body, the nervous system is of great importance. Injury or some diseases can damage nerves and impair their functions. Tissue engineering can be used to rebuild the damage parts of the nerves by constructing neural circuits. Such circuits can be built by guiding the neural cells along a well-defined micropattern. Axon branching is also essential to the signaling and functioning of the cells. Therefore, neural cells should exhibit axonal outgrowths and the ability to grow along micropatterns so that the functional neural circuits can be built.

In this study, an initial step towards building neural circuits will be explored by attempting to confine neural cells on micropatterns. The patterns will be fabricated from gold-based metallic glass. Metallic glass is an amorphous metal with atoms arranged randomly unlike normal metals. Metallic glass has yet to be excessively studied in the biological field. This will be the first stepping stone towards utilizing this material. Metallic glass has good processability as well as good conductivity. The conductivity will be beneficial

for electrical stimulation that can be performed with neural cells. The micropatterns will be subjected to cell culture using the cell line called Neuro2A.

This investigation will be conducted in hopes that fully functional neural circuits can be constructed in the future. The utilization of gold-based metallic glass is to study the ease of microfabrication as well as its ability to be used in neural cell culturing. The neural cells cultured on the micropatterned gold-based metallic glass will be observed to see the cell adhesion and the extent of axon branching. It is believed that the information from this study will be used as the building blocks for constructing neural circuits.

เนื้อเรื่อง (Main Body)

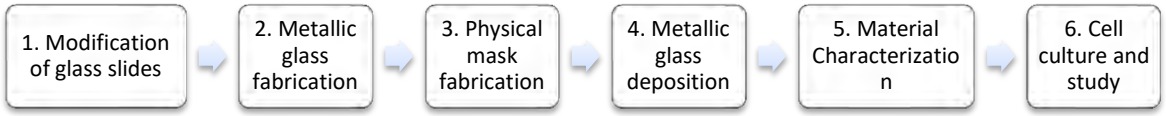
วัตถุประสงค์ (Objectives)

1. Explore the application of gold-based metallic glass in biological aspect
2. Pioneer in the field of neural circuit construction for nerve regeneration, nerve repair, and neural induction using gold-based metallic glass
3. Determine the optimum width and thickness for the micropatterned stripes containing metallic glass that will produce the most favorable result
4. Investigate the effects from the incorporation of electronic probes to induce electrical field on the neural cell-cultured micropatterned gold-based metallic glass

วิธีดำเนินการวิจัย (Materials & Methods)

This study is divided primarily into two main parts. The first part is the determination of the optimal size and thickness of the micropattern for cell adhesion and axon branching. The second part is to use the result from the first part to create the micropattern that will result in the best outcome.

The processes can be outline as follow:



1. Modification of glass slides

Glass slides will be use as substrate for micropatterning in this study. They have been selected due to their optical clarity and they are commercially available. The size of the glass slide to be used for the substrate will be 1.5 x 1.5 cm^2. Before micropatterning, these glass

slides have to be treated to block cell adhesion so that cells will be guided by the micropatterns. The method for glass surface modification is adopted from Tan et. al study of substrate wettability. Pluronic surfactants will be use to prevent cell adhesion. Pluronic surfactants are block polymers of polyethylene glycol-polypropylene glycol-polyethylene glycol. The polypropylene segment is hydrophobic and is responsible for binding to the substrate. The two tails of polyethylene protrudes out and blocking protein adsorption and cell adhesion. Since the attachment part of the Pluronic surfactants is hydrophobic, it is required that the substrate's is also hydrophobic. However, glass is hydrophilic. The hydrophilic glass can be altered to hydrophobic by using the process of silanization, which is also adopted from Tan et al. Silanization is to coat the glass substrate with alkoxysilane molecules. This process will allow the formation of the bond between the glass (mineral component) and the Pluronic surfactant (organic component).

2. Metallic glass fabrication

The fabrication of the gold-based metallic glass from the elements for the micropattern is a crucial step. The formula of the metallic glass is Au49Ag5.5Pd2.3Cu26.9Si16.3 with the composition in atomic percentages. To form the gold-based metallic glass—gold, silver, palladium, copper and silicon, are put into an arc-melter. The individual elements are weighed according to their atomic percentages. They are then subjected to the heating by electric arc in the melter to form gold-based metallic glass.

The basic principle to create an alloy from the arc-melter is as follows:

1) Before the application of the arc, the arc plate has to be polished by brasso acetone alcohol sand paper to clean the surface. Vacuum grease is then applied to prevent the air from leaking into the system. The vacuum pump is utilized because the system has to be oxygen free in order to fabricate the alloy with the highest purity.

2) When placing the elements that constitute the alloy, the larger piece should be covering the smaller pieces to prevent lost of material when the arc is applied.

3) Along with the components, Ti Getters are also put on the plate to get all the oxygen prior to and after the arcing process.

4) The copper plate has to be cooled with running water to prevent the plate from melting and argon flow is also added to make the argon atmosphere inside the system.

5) For the arcing process, the tungsten rod is brought in close together to create a spark and flame. Arc getter is then created to absorb the oxygen residue before and after the application of the arc to create the desired alloy.

6) When arcing, the material has to be flipped to achieve a homogeneous state of the metal alloy. The flipping is done 3-4 times.

7) Once the metal alloy is fabricated, the electrical generator has to be turned off before bringing out the finished metal alloy.

3. Physical mask fabrication

Physical mask is needed to create the gold-based metallic glass micropattern by blocking some parts of the substrates from metallic glass deposition. There are two methods for the physical mask fabrication. One is to manually create the pattern and another is to order the pattern from a mask producer.

For the first part of the experiment which is to determine the optimum width and thickness of the micropattern. The thickness of the micropattern is controlled by the deposition process, which will be talked about in the next section. The pattern of the mask is designed to have varying width. The extent of cell adhesion to different width will be observed to determine the optimal point. The size of the Neuro 2A cells are approximately 10μm when attached to a substrate. The intention is to see whether the neural cells prefer the micropattern larger than their size or smaller than their size.

After the determination of the optimal width and thickness, the pattern of the mask will no longer have varying width. Three width sizes will be chosen from the first part of the experiment so that the cell adhesion and axon outgrowth can be observed. Subsequently, chemicals cues or electrical stimulation can be induced to the neural cells.

4. Metallic glass deposition

To get the thin film of amorphous metal on the substrate the method of deposition that will be used is thermal evaporation. This method is a physical vapor deposition that is used to deposit the target material by condensation after the vaporization. In this case, the vapor of the metallic glass is created by resistive heating of the material on the boat or crucible. The vapor then condenses onto the substrate surface, as well as the vacuum chamber walls, to form the thin film of amorphous metal. This method utilizes high vacuum so the contaminations are reduced. Because of the long mean free path of the molecules, it is very directional. The downside is that shadowing effect may occur. Although, the step coverage may not be good, the deposition rate is high when compare to other processes. The ease of using the process is that the source does not need to be created. The thickness of the amorphous metal film can also be controlled by changing the mass of the metallic glass source. Two thicknesses of 10 μm and 30 μm will be investigated. It was found in the previous study that for thicknesses exceeding 50 μm, the light will not pass through and that the amorphous metal thin film peels off easily. Therefore, it is seen to be appropriate to use thicknesses of 10 μm and 30 μm to see its effects on neural cells.

The physical mask will be attached to the substrate and the metallic glass will be deposited on the substrate through the pattern.

5. Characterization

Succeeding the microfabrication of the pattern, characterization of the amorphous metal thin film has to be investigated. The characterization will involve the use of several techniques, including x-ray diffraction (XRD), atomic force microscopy (AFM), and scanning electron microscope (SEM) along with the energy dispersive x-ray spectroscopy (EDX).

X-ray diffraction (XRD) is a non-destructive technique that is used to determine the crystalline structure and chemical composition of the amorphous metal thin film created. Incident x-ray is diffracted by the sample and is detected by the detector. The incident angle is θ while the diffracted angle is 2θ.The XRD thermogram of this amorphous metal of the same composition, Au49Ag5.5Pd2.3Cu26.9Si16.3 has been shown to have broad maxima, which is typical for amorphous material [4]. The peak is around 2θ = 40° [4]. This technique will be used to determine whether the amorphous metal thin film is deposited as the micropattern.

6. Cell culture and study

Neuro 2A cells are used in this study after the fabrication and characterization of the material is done. Cell culture must be done very carefully since contamination of the cells is fairly easy. The cells are seeded onto a 10cm-culture plate and the media is changed every 2 days. After 4 days, sub-culturing can be done. Sub-culturing of cells is to divide the cells from one plate into 4 plates to generate more cells. Minimum Essential Medium (MEM), is one of the most widely used of all synthetic cell culture media. It provides the minimal requirements for the growth of cells, such as amino acids and sugar. Fetal bovine serum (FBS) is used for cell culture because it contains growth factors. Pen-strep is a type of antibiotic and is included into the media solution to prevent contamination. L-glutamine is added due to its role in protein synthesis, as well as source of cellular energy.

ผลการวิจัย (Results)

Modification of glass slides and metallic glass fabrication

The preparation of silanized glass is through the following process:

1) The glass slides are cleaned with solution of 15% H_2O_2 and 15% NH_4OH in water at 60°C for 30min.
2) It is then immersed in 5% dimethyl dichlorosilane in dichlorobenzene for approximately 10s.
3) Next, the glass is rinsed with acetone then water and blown dried.
4) Prior to use, the silanized glass is cleaned using the UV.

The silanized glass is then subjected to Pluronic surfactant coating. This is done by immersing the silanized glass in 0.2% (w/v) Pluronic in phosphate-buffered saline (PBS) for 1h [12]. The glass is then rinsed with water without allowing the surface to dry [12].

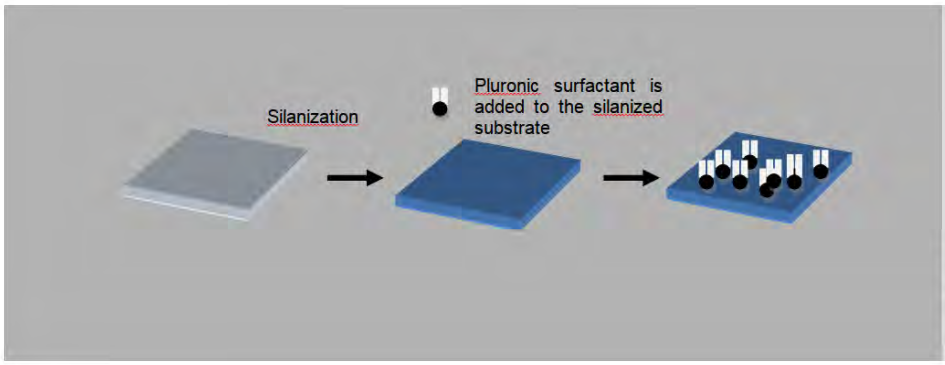


Figure 1

Glass modification processes are show as schematic drawing.

Physical mask fabrication

To manually create the pattern, two transparencies will be used. The distance between the two transparencies will be the width of the mask micropattern. The distance will be created by placing extremely thin material such as foil with known thickness between the transparencies. After the two transparencies are in place, they will be fixed.

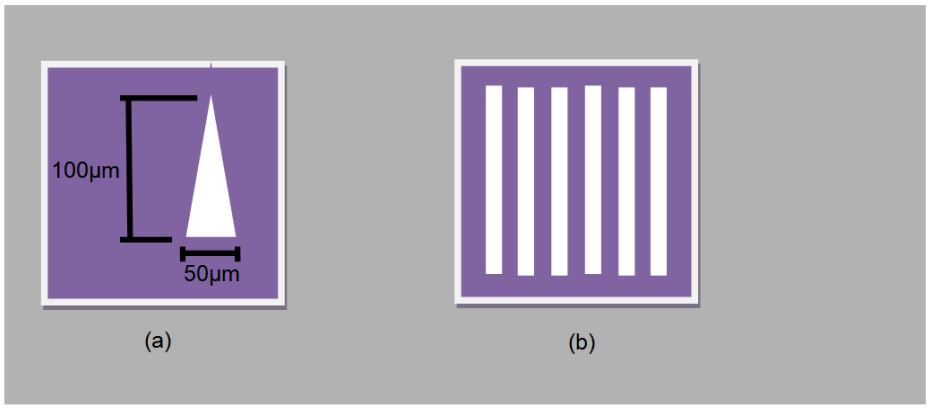


Figure 2

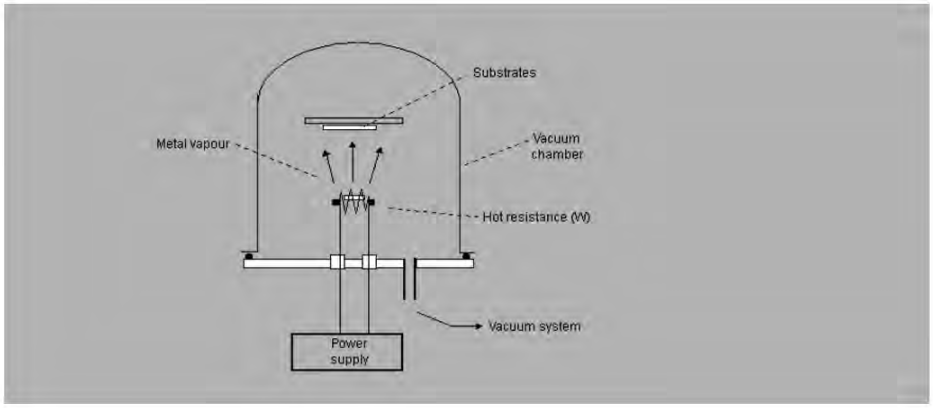
(a) Mask pattern to determine optimal width. (b) Mask pattern after the optimal width has been established.

Metallic glass deposition

The schematic diagram of the method is shown in Figure 3. In this case, the vapor of the metallic glass is created by resistive heating of the material on the boat or crucible. The vapor then condenses onto the substrate surface, as well as the vacuum chamber walls, to form the thin film of amorphous metal. This method utilizes high vacuum so the contaminations are reduced. Because of the long mean free path of the molecules, it is very directional.

Figure 3

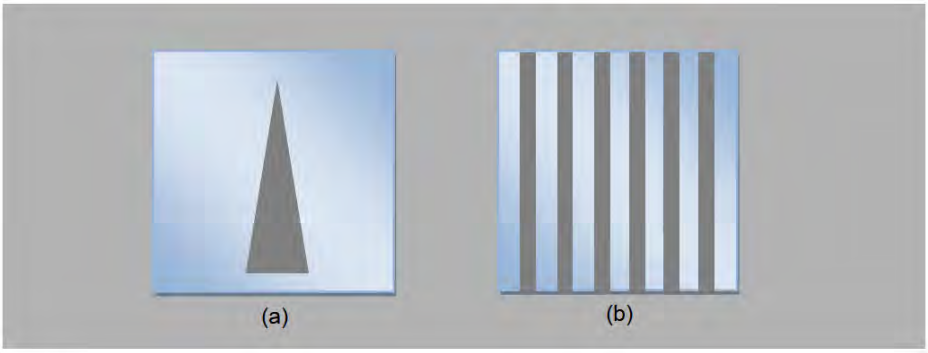
The schematic diagram of the thermal evaporation is shown here.



The physical mask will be attached to the substrate and the metallic glass will be deposited on the substrate through the pattern. Illustrations of the finished products are shown in Figure 4 (a) and (b). In Figure 4 (a), the mask is from the first part for the determination of the optimum width and thickness and in (b), the mask use is with stripe patterns.

Figure 4

Schematic diagram showing the patterned substrate with metallic glass in grey color. (a) is from the first part of the study (b) is the stripe pattern with the optimum width



Characterization

Atomic force microscopy (AFM) technique is used to generate the 3D image and determine the surface property by scanning of a probe. This technique is non-destructive and can also be used in situ. The basic principle of this technique is to use the interaction force between the probe and the surface. The probe is scanned across the surface to gain information on the surface of the material. The laser is used to measure the amount of bending of the probe in the contact mode. This technique will be used to observe the surface of the amorphous metal thin film micropattern on the substrate.

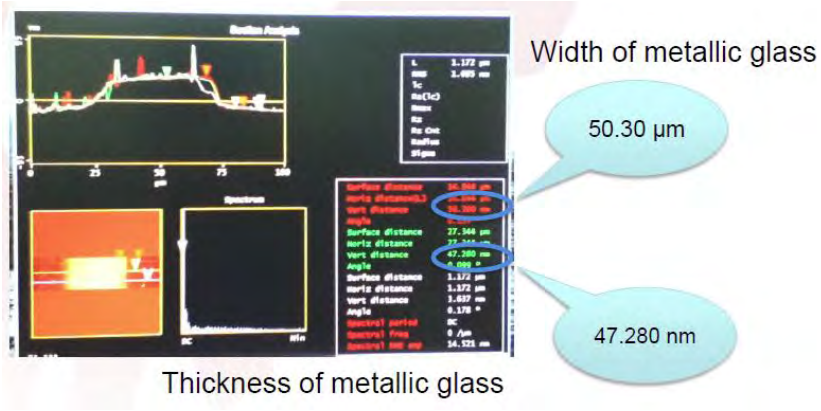


Figure 5 Characterization of metallic glass on substrate using AFM

Scanning electron microscope (SEM) is a type of electron microscope that provides image of the topography of the sample by scanning around it. Auger, secondary, back-scattered electrons and characteristic x-rays are generated when the electron beam hits the material. The secondary electrons are often detected due to the fact that they carry information about the surface morphology of the material. Characteristic x-rays can also be detected. The detection of these x-rays contributes to the working principle of energy dispersive x-ray technique. The characteristic x-rays are unique to each material; therefore, the detection of this will provide information of the composition of the material. SEM will be used to observe the surface morphology of the deposited amorphous metal and EDX will be used to determine the composition.

Cell culture and study

The outline of the steps for cell sub-culture is as follow:

1. Remove all media
2. Wash with 5ml 1X FBS one time then remove all solution
3. Add 1 ml (warm) 0.25% trypsin EDTA
4. Incubate at 37°C for 2 min (Cells will be damaged if left for too long)
5. Observe cells to confirm they are not adhered to plate (spherical single cells)
6. Stop trypsin reaction by media 2-4 ml (at least two times the amount of trypsin added)
7. Resuspend to get single cells
8. Put all the solution into 15 ml tube
9. Centrifuge at 1000 rpm – 5 min
10. Discard suspension then resuspend with 1ml media
11. Transfer the cells to the dish that already has 10ml media (6 wells use 2ml)
12. Agitate in 8 directions

Table 1

This table shows media components and the percentage of FBS that can be used.

Components/%FBS	10%FBS	5%FBS	0%FBS
MEM	36 ml	38 ml	40 ml
FBS	4 ml	2 ml	0 ml
Pen-Strep	400 µl	400 µl	400 µl
L-glutamine	400 µl	400 µl	400 µl

Once the material is fabricated the cells from the culture plate will be divided in approximately equal number to be put into a 6-well-plate to observe the cell adhesion and axon outgrowths on the substrate micropatterned with metallic glass.

Both the extent of cell adhesion and axon branching is observe qualitatively using inverted microscope. Cell counting is a way to measure the amount of neural cells adhesion. Cells can be counted using hemocytometer. The hemocytometer is made of glass with known dimension chambers. The cells can then be counted in the specific volume of fluid.

Adhesion and proliferation tests

Micropatterned metallic glass on substrate were displayed in Figure 6. From the adhesion test result in Figure 7 and 8, Neuro2A cells are able to adhere to all the surface with the most adhered cells and the least adhered cells being the positive and the negative control respectively. The adhered cells in the negative control showed comparable adherence to the glass substrate.

Figure 6 Pictures of metallic glass micropattern from inverted fluorescence microscope. Width of metallic glass micropattern were 50 – 100 µm

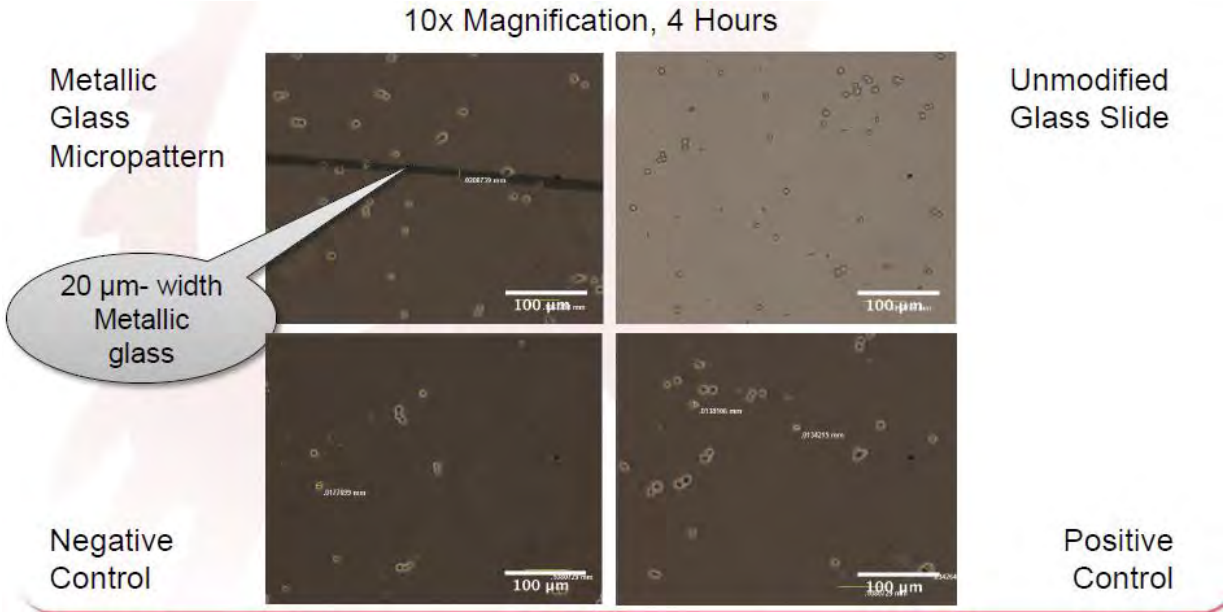


Figure 7 Adhesion test (4 hours). 10X magnification, 4 hours

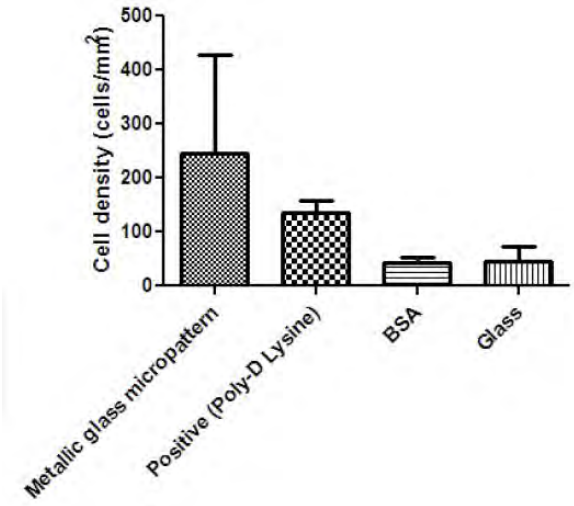


Figure 8 Summary of adhesion test

From the proliferation test in Figure 9 and 10, there was not specific pattern of cell proliferation on metallic glass. However, cell density on metallic glass increased. This finding indicated that cell proliferation was magnified on metallic glass.

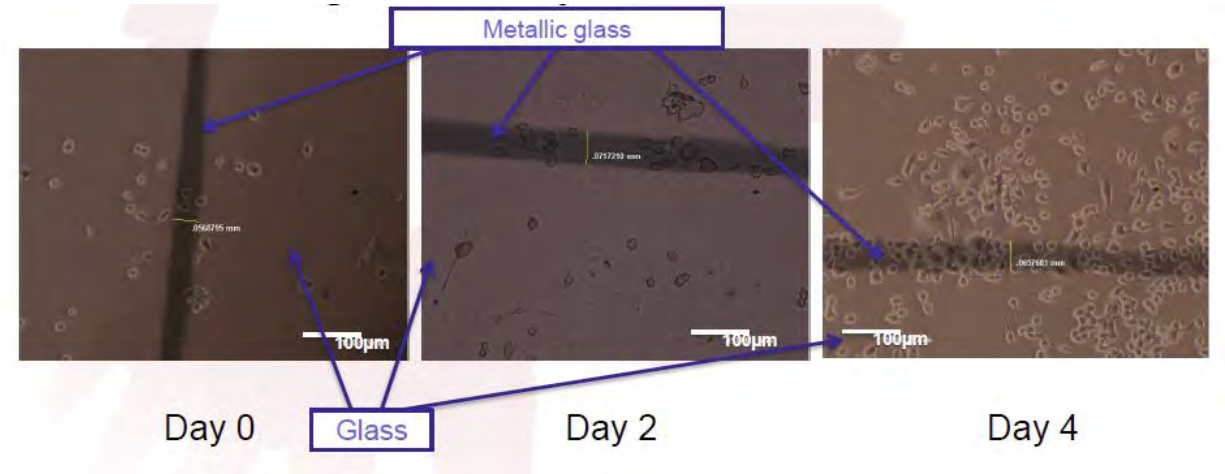


Figure 9 Metallic glass micropatterned with/without surface treatment

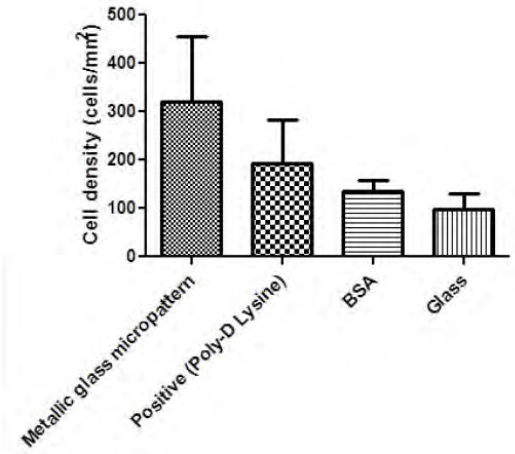


Figure 10 Summary of proliferation test

อภิปราย/วิจารณ์ (Discussion) ผลการทดลอง

From results, it can be observed that this cell line, Neuro2A, was not substrate specific. These cells adhered even to the negative control but they proliferate better on metallic glass micropattern. Due to the time restriction and equipment availability, only a simple process of photolithography was done. Further investigation may include the usage of microcontact printing and the liftoff process as well as an in-depth investigation on the morphology of the designated gold-based metallic glass. The deposition of the gold-based metallic glass was performed using thermal evaporation. Other alternative ways of deposition including electron-beam sputtering, RF sputtering, magnetron sputtering, plasma-enhanced chemical vapor deposition, low pressure chemical vapor deposition, and microcontact printing may be utilized as they may posed as potential mean to generate a homogenous high-quality deposit.

In this study, Pluronic is used as a sole anti-cell adhesive surfactant. It is not known about the surface interaction between Pluronic surfactant and the gold-based metallic glass although there were only investigations regarding Pluronic surfactant and glass substrate. This would a limitation to this study.

Neuro 2A was cultured onto the patterned substrate. If the substrate cell culture was a success, an incorporation of electrical probes was included in this study to observe the conductance of the neural circuit and the differentiation of the neuro 2A cell line.

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ประวัตินักวิจัยและคณะ

หัวหน้าโครงการวิจัย รองศาสตราจารย์ ดร.ศักนัน พงศ์พันธุ์ผู้ภักดี

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7.3.11. Saleewong T, Srikiatkhachorn A, Maneepark M, Chonwerayuth A, Bongsebandhu-phubhakdi S. Computational approach to long-term potentiation in hippocampal CA1 area describes the efficacy of stimulation patterns. ***Asian Biomedicine.*** 2013; 7: 347-356.

7.3.12. Junsre U, Bongsebandhu-phubhakdi S. ASICs Alteration by pH Change in Trigeminal Ganglion Neurons. ***Journal of Physiology and Biomedical Sciences.*** 2014; 27: 20-25.

7.3.13. Vibulyaseck S, Bongsebandhu-phubhakdi S, le Grand SM, Srikiatkhachorn A. Potential risk of dihydroergotamine causing medication-overuse headache; preclinical evidence. ***Asian Biomedicine.*** 2014; 8: 323-331.

7.3.14. Hansrivijit P, Vibulyaseck S, Maneepark M, Srikiatkhachorn A, Bongsebandhu-phubhakdi S. Cortical spreading depression increases NR2A/NR2B ratio by altering numbers of nr2a

and nr2b subunit-containing nmda receptors in the hippocampus. ***The Journal of Headache and Pain.*** 2014; 15 (Suppl 1): F8.

7.3.15. Hansrivijit P, Vibulyaseck S, Maneepark M, Srikiatkhachorn A, Bongsebandhu-phubhakdi S. GluN2A/B ratio elevation induced by cortical spreading depression: Electrophysiological and quantitative studies of the hippocampus. ***Journal of Physiological sciences.*** 2015; 65 (Suppl 2): S3-S10.

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7.3.17. Saleeon W, Jansri U, Srikiatkhachorn A, Bongsebandhu-phubhakdi S. The estrous cycle modulates voltage-gated ion channels in TG neurons. ***Journal of Physiological sciences.*** 2015; 65 (Suppl 2): S-A174.

7.3.18. Saleeon W, Jansri U, Srikiatkhachorn A, Bongsebandhu-phubhakdi S. Estrous cycle induces peripheral sensitization in TG neurons: An animal model of menstrual migraine. ***Journal of Medical Association Thailand.*** 2016; 99: 206-212.

7.3.19. Niyomnaitham D, Vilaisaktipakorn P, Wongjarupong N, Suksiriworaboot T, Bongsebandhu-phubhakdi S. Interactive laboratory classes enhance neurophysiological knowledge in medical students. ***Advances in Physiology Education.*** 2018; 42: 140-145.

7.3.20. Kwankajonwong N, Ongprakobkul C, Qureshi SP, Watanatada P, Thanprasertsuk S, Bongsebandhu-phubhakdi S. Attitude but not self-evaluated knowledge correlates with academic performance in physiology in Thai medical students. ***Advances in Physiology Education.*** 2019; accepted.

7.4. งานวิจัยที่กำลังทำ: ชื่อข้อเสนอการวิจัย แหล่งทุน และสถานภาพในการทำวิจัยว่าได้ทำการวิจัยลุล่วงแล้วประมาณร้อยละเท่าใด

7.4.1. Branching and adhesion study of neurons on metallic glass
แหล่งทุน โครงการวิจัยและนวัตกรรมเชิงยุทธศาสตร์และโครงสร้างพื้นฐาน ปี 2561
สถานภาพในการทำวิจัย ร้อยละ 70

7.4.2. Genetic variation and mutation of potassium channels in sudden unexplained death cases
แหล่งทุน ทุนวิจัยรัชดาภิเษกสมโภช คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
สถานภาพในการทำวิจัย ร้อยละ 50

7.4.3. Identification and correlation between calcitonin gene related peptide expression in glottic cancer
แหล่งทุน ทุนวิจัยรัชดาภิเษกสมโภช คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
สถานภาพในการทำวิจัย ร้อยละ 80

7.4.4. Integration of mathematical model in SPECT imaging for epilepsy evaluation
แหล่งทุน ทุนงบประมาณแผ่นดิน ปี 2560
สถานภาพในการทำวิจัย ร้อยละ 60

อาจารย์ผู้วิจัยร่วม อาจารย์ ดร.บุญรัตน์ โล่ห์วงศ์วัฒน

1. ชื่อ - นามสกุล (ภาษาไทย) ดร.บุญรัตน์ โล่ห์วงศ์วัฒน ผู้ช่วยศาสตราจารย์
   ชื่อ - นามสกุล (ภาษาอังกฤษ) Dr. Boonrat Lohwongwatana, Ph.D., Assistant Professor
2. เลขหมายบัตรประจำตัวประชาชน 3-1002-02837-54-1
3. ตำแหน่งปัจจุบัน ผู้ช่วยศาสตราจารย์ระดับ A-4
4. หน่วยงาน ภาควิชาวิศวกรรมโลหการ คณะวิศวกรรมศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
   โทรศัพท์ 0-2218-6939 โทรสาร 0-2218-6942
   e-mail: boonrat@gmail.com
5. ประวัติการศึกษา
   - M.S. (พ.ศ.2545) และ Ph.D. (พ.ศ.2550) Materials Science and Engineering
     California Institute of Technology, Pasadena, CA, ประเทศสหรัฐอเมริกา
   - B.E. (พ.ศ.2543) Materials Science and Engineering (Honors)
     Northwestern University, Evanston, IL, ประเทศสหรัฐอเมริกา
   - มัธยมศึกษา จาก โรงเรียนสวนกุหลาบวิทยาลัย (พ.ศ.2538)
6. สาขาวิชาการที่มีความชำนาญพิเศษ (แตกต่างจากวุฒิการศึกษา) ระบุสาขาวิชาการ
   Precious Metals, Solder Materials, Alloys Development, Thermodynamics Modeling, Nano-materials
7. ประสบการณ์ที่เกี่ยวข้องกับการบริหารงานวิจัยทั้งภายในและภายนอกประเทศ โดยระบุสถานภาพในการทำการวิจัยว่าเป็นผู้อำนวยการแผนงานวิจัย หัวหน้าโครงการวิจัย หรือผู้ร่วมวิจัยในแต่ละผลงานวิจัย
   7.1 ผู้อำนวยการแผนงานวิจัย : ชื่อแผนงานวิจัย
   ไม่มี
   7.2. หัวหน้าโครงการวิจัย : ชื่อโครงการวิจัย

1.	การพัฒนาโลหะผสมที่ไม่มีตะกั่วเพื่อการบัดกรีในงานอุตสาหกรรม อิเล็กโทรนิกส์	ดร.บุญรัตน์ โล่ห์วงศ์วัฒน รศ.ดร.กอบบุญ หล่อทองคำ	สำนักงานกองทุนสนับสนุนการวิจัย (สกว.) TRF-MAG	2552-2554 (600,000)
2.	การปรับปรุงกระบวนการเชื่อมบัดกรีแบบเจ็ดบอนด์ดิ้งในหัวอ่านฮาร์ดดิสค์เพื่อปรับค่าระดับความเอียงของหัวอ่าน	อ.ดร.บุญรัตน์ โล่ห์วงศ์วัฒน	Western Digital (Thailand) Co., Ltd.	2554 (328,670)
3.	เทคโนโลยีการตัด ขัดใส และขัดแบบระเอียด (การวิเคราะห์ความเค้นบนผิวในกระบวนการแปรรูปแผ่น AlTiC)	อ.ดร.บุญรัตน์ โล่ห์วงศ์วัฒน ผศ.ดร.ธาชาย เหลือวรานันท์	Western Digital (Thailand) Co., Ltd.	2554-2555 (740,350)
4.	การขึ้นรูปแบบเทอร์โมพลาสติกของรัตนโลหะที่มีทองคำเป็นส่วนประกอบหลัก	อ.ดร.บุญรัตน์ โล่ห์วงศ์วัฒน	สำนักงานกองทุนสนับสนุนการวิจัย (สกว.) ร่วมกับสมาคมผู้ค้าอัญมณีไทยและเครื่องประดับ	2554-2556 (400,000)
5.	การสังเคราะห์อนุภาคระดับนาโนโดยใช้รัตนโลหะทอง	อ.ดร.บุญรัตน์ โล่ห์วงศ์วัฒน	สำนักงานกองทุนสนับสนุนการวิจัย (สกว.)	2552-2554 (480,000)

6.	การออกแบบพัฒนาโลหะผสมที่ไม่มีตะกั่วเพื่อการบัดกรีในงานอุตสาหกรรมโดยใช้กระบวนการวิเคราะห์แบบ Evolutionary Algorithm	อ.ดร.บุญรัตน์ โล่ห์วงศ์วัฒน รศ.ดร.กอบบุญ หล่อทองคำ	สำนักงานกองทุนสนับสนุนการวิจัย (สกว.) TRF-MAG	2551-2553 (600,000)

7.3. งานวิจัยที่ทำเสร็จแล้ว : ชื่อผลงานวิจัย ปีที่พิมพ์ การเผยแพร่ และแหล่งทุน (อาจมากกว่า 1 เรื่อง)

1. **Lohwongwatana B**, Lewan A, Thipayarat K, Akara-Apipokee N, and Nisaratanaporn E. Metals by design: From ultra-hard *in-situ* nano-composite gold jewelry articles to silver alloys castable in silicone mold. **Invited speaker** to the 26th SANTA FE SYMPOSIUM for jewelry manufacturing technology, Albuquerque, NM. May 20-23, 2012.
2. Piyavatin P, Lothongkum G, and **Lohwongwatana B.** Characterization of eutectic Sn-Cu solder alloy properties improved by additions of Ni, Co & In MP Materials Testing, 06/2012, page 383-389.
3. Thongprasom K, Suvanpiyasiri C, Wongsa A, Iamaroon A, Korkij W, **Lohwongwatana B**, Sinpitaksakul S, and Nakpipat P. Nickel - Induced Oral Pemphigus Vulgaris -Like Lesions Acta Stomatol Croat. 2011;45(3):202-208.
4. **Lohwongwatana B**, and Nisaratanaporn E. On Hardness **Invited speaker** to the 24th SANTA FE SYMPOSIUM for jewelry manufacturing technology, Albuquerque, NM. May 16-19, 2010.
5. **Lohwongwatana B**, Nisaratanaporn E and Holstein J. Alloys By Design – Knowing the Answer before Spending money **Invited speaker** to the 22st SANTA FE SYMPOSIUM for jewelry manufacturing technology, Albuquerque, NM. May 18-21, 2008.
6. Suh JY, **Lohwongwatana B**, Garland C, Conner R, Johnson WL, and Suh D. Novel Thermoplastic Bonding Using Bulk Metallic Glass Solder SCRIPTA MATERIALIA, 59 (2008) 905-908. **(Impact Factor 2.481)**
7. **Lohwongwatana B**, Schroers J and Johnson WL. Hard 18K and .850 Pt. alloys that can be processed like plastics or blown like glass **Invited speaker** to the 21st SANTA FE SYMPOSIUM for jewelry manufacturing technology, Albuquerque, NM. May 20-23, 2007. **(Received Outstanding Technical Presentation Award)**
8. **Lohwongwatana B**, Schroers J and Johnson WL. Strain rate induced crystallization in bulk metallic glass-forming liquid PHYSICAL REVIEW LETTERS 96 (7): Art. No. 075503 FEB 24 2006 **(Impact Factor 7.072)**
9. Schroers J, **Lohwongwatana B**, Johnson WL and Peker A. Precious bulk metallic glasses for jewelry applications MATERIALS SCIENCE AND ENGINEERING A-STRUCTURAL MATERIALS PROPERTIES MICROSTRUCTURE AND PROCESSING 449: 235-238 MAR 25 2007 **(Impact Factor 1.490)**
10. Xu DH, **Lohwongwatana B**, Duan G, Johnson WL and Garland C. Bulk metallic glass formation in binary Cu-rich alloy series - Cu100-xZrx (x=34, 36 38.2, 40 at.%) and mechanical properties of bulk Cu64Zr36 glass ACTA MATERIALIA 52 (9): 2621-2624 MAY 17 2004. **(Impact Factor 3.729)**

11. Udomlertpreecha S, Pavasant P, **Lohwongwatana B.** Surface modification of titanium alloys using alumina particles blasting for biomedical applications Advanced Materials Research 983: 135-140 2014. **(Impact Factor 0.140)**
12. Thumsoontorn S, Kuimalee S, Kuntalue B, Pintasiri S, **Lohwongwatana B.** Microstructure and Direct Measured Micro-strain by TEM of Hot Iso-static Pressed Alumina-Titanium Carbide (Al_2O_3-TiC) Composite Advanced Materials Research 983: 156-160 2014. **(Impact Factor 0.140)**
13. Punyaratabandhu T, **Lohwongwatana B**, Puncreobutr C, Kosiyatrakul A, Veerapan P, Luenam S. Patient-Matched Entire First Metacarpal Prosthesis in Treatment of Giant Cell Tumor of Bone. Case Rep Orthop. 2017;2017:4101346.
14. Luenam S, Kosiyatrakul A, Hansudewechakul C, Phakdeewisetkul K, **Lohwongwatana B**, Puncreobutr C. The Patient-Specific Implant Created with 3D Printing Technology in Treatment of the Irreparable Radial Head in Chronic Persistent Elbow Instability. Case Rep Orthop. 2018 Oct 23;2018:9272075.
15. Luenam S, Vongvanichvathana A, Kosiyatrakul A, Kongphanich C, Chanpoo M, Koonchornboon T, Phakdeewisetkul K, **Lohwongwatana B**, Puncreobutr C. Matching precision of the reverse contralateral radial head in generating of the individualized prosthesis from the surface registration in tuberosity-neck and in tuberosity-diaphysis. J Orthop Surg (Hong Kong). 2019 Jan-Apr;27(1):2309499018821774.

7.4. งานวิจัยที่กำลังทำ : ชื่อข้อเสนอการวิจัย แหล่งทุน และสถานภาพในการทำวิจัยว่าได้ทำการวิจัยลุล่วงแล้วประมาณร้อยละเท่าใด

			Funding Agencies	Funding	สถานะ
1.	การพัฒนาโลหะผสมที่ไม่มีตะกั่วเพื่อการบัดกรีในงานอุตสาหกรรม อิเล็กโทรนิกส์	ดร.บุญรัตน์ โล่ห์วงศ์วัฒน รศ.ดร.กอบบุญ หล่อทองคำ	สำนักงานกองทุนสนับสนุนการวิจัย (สกว.) TRF-MAG	2552-2554 (600,000)	เสร็จสิ้น 100% ปิดโครงการแล้ว
2.	การปรับปรุงกระบวนการเชื่อมบัดกรีแบบเจ็ทบอนด์ดิ้งในหัวอ่านฮาร์ดดิสค์ เพื่อปรับค่าระดับความเอียงของหัวอ่าน	อ. ดร. บุญรัตน์ โล่ห์วงศ์วัฒน	Western Digital (Thailand) Co., Ltd.	2554-2555 (328,670)	เสร็จสิ้น 100% ระหว่างปิดโครงการ (ส่ง final report แล้ว)
3.	เทคโนโลยีการตัด ขัดใส และขัดแบบระเอียด (การวิเคราะห์ความเค้นบนผิวในกระบวนการแปรรูปแผ่น AlTiC)	อ. ดร. บุญรัตน์ โล่ห์วงศ์วัฒน ผศ. ดร. ธาชาย เหลือวรานันท์	Western Digital (Thailand) Co., Ltd.	2554-2555 (740,350)	เสร็จสิ้น 100% ระหว่างปิดโครงการ (ส่ง final report แล้ว)
4.	การขึ้นรูปแบบเทอร์โมพลาสติกของรัตนโลหะที่มีทองคำเป็นส่วนประกอบหลัก	อ. ดร. บุญรัตน์ โล่ห์วงศ์วัฒน	สำนักงานกองทุนสนับสนุนการวิจัย (สกว.) ร่วมกับสมาคมผู้ค้าอัญ	2554-2556 (400,000)	90%

			มณีไทยและ เครื่องประดับ		
5.	การสังเคราะห์อนุภาคระดับนาโนโดยใช้รัตนโลหะทอง	อ. ดร. บุญรัตน์ โล่ห์วงศ์วัฒน	สำนักงานกองทุนสนับสนุนการวิจัย (สกว.)	2552-2554 (480,000)	ปิดโครงการแล้ว
6.	การออกแบบพัฒนาโลหะผสมที่ไม่มีตะกั่วเพื่อการบัดกรีในงานอุตสาหกรรม โดยใช้กระบวนการวิเคราะห์แบบ Evolutionary Algorithm	ดร.บุญรัตน์ โล่ห์วงศ์วัฒน รศ.ดร.กอบบุญ หล่อทองคำ	สำนักงานกองทุนสนับสนุนการวิจัย (สกว.) TRF-MAG	2551-2553 (600,000)	ปิดโครงการแล้ว

ภาคผนวก (Appendix)

บทความที่เผยแพร่ในวารสารวิชาการนานาชาติ

- Wongjarupong N, Niyomnaitham D, Vilaisaktipakorn P, Suksiriworaboot T, Bongsebandhu-phubhakdi S. Interactive laboratory classes enhance neurophysiological knowledge in medical students. ***Advances in Physiology Education.*** 2018; 42: 140-145.
- Kwankajonwong N, Ongprakobkul C, Qureshi SP, Watanatada P, Thanprasertsuk S, Bongsebandhu-phubhakdi S. Attitude but not self-evaluated knowledge correlates with academic performance in physiology in Thai medical students. ***Advances in Physiology Education.*** 2019; accepted.

บทความที่รอการเผยแพร่ในวารสารวิชาการนานาชาติ

- Haji-a-sa N, Thanprasertsuk S, Bongsebandhu-phubhakdi S. Serotonin depletion amplifies excitability of trigeminal ganglia nociceptive neuron: a patch clamp recording study. ***Journal of Physiological Sciences.*** 2019; on review.
- Leesutipornchai T, Ratchataswan T, Vivatvakin S, Ruangritchankul K, Keelawat S, Kerekhanjanarong V, Bongsebandhu-phubhakdi S, Mahattanasakul P. EGFR cut-off point for prognostic impact factor in laryngeal squamous cell carcinoma. ***JAMA Otolaryngology–Head & Neck Surgery.*** 2019; on review.

บทคัดย่อ การนำเสนอผลงานวิชาการระดับนานาชาติ

- Ongprakobkul C, Kwankajonwong N, Bongsebandhu-phubhakdi S, Thanprasertsuk S. Physiology Teaching Time Proportion Affects Student's Attitude and Their Self-evaluation Rating. 2019 World Federation for Medical Education World Conference (WFME 2019). Seoul, Korea, April 7th – 10th, 2019. (Poster presentation)
- Kwankajonwong N, Ongprakobkul C, Thanprasertsuk S, Bongsebandhu-phubhakdi S. Influences of attitude and self-evaluation rating to academic performance in physiology learning: a quantitative study in Thai medical student. 2019 World Federation for Medical Education World Conference (WFME 2019). Seoul, Korea, April 7th – 10th, 2019. (Poster presentation)
- Haji-a-sa N, Thanprasertsuk S, Bongsebandhu-phubhakdi S. 5-HT depleted and KCl-induced CSD rats provide difference electrophysiological properties of neurons recorded by patch-clamp techniques. 30th Edition of International Conference on Neurology & Neuroscience Week. Singapore, May 27th – 28th, 2019. (Selected oral presentation)
- Wichit P, Thanprasertsuk S, Bongsebandhu-phubhakdi S. The alteration of plasma monoamine neurotransmitters level related to psychiatric, sleep, and sexual problems in Parkinson's disease patients. 30th Edition of International Conference on Neurology & Neuroscience Week. Singapore, May 27th – 28th, 2019. (Selected oral presentation)

- Mahattanasakul P, Ruangritchankul K, Bongsebandhu-phubhakdi S, Vivatvakin S, Ratchataswan T, Leesutipornchai T, Keelawat S, Kerekhanjanarong V. Clinicopathological and prognostic value of EGFR expression in laryngeal squamous cell carcinoma. 5th Congress of European ORL-Head & Neck Surgery. Brussels, Belgium, June 29th – July 3rd, 2019. (Poster presentation)

บทคัดย่อ การนำเสนอผลงานวิชาการระดับชาติ

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ภาพพิธีรับรางวัล

- รางวัลชนะเลิศ โล่รางวัลและทุนการศึกษา จากการนำเสนอแบบบรรยาย ในการประชุม UAMC 2018 "ตัวแบบเชิงคณิตศาสตร์สำหรับสารรังสีไอโอดีน 131I เพื่อการรักษามะเร็งต่อมไทรอยด์ชนิดพาพิลลารีด้วยการวิเคราะห์ภาพฉาย SPECT/CT; A Mathematical Model of Radioiodine (131I) Therapy in Papillary Thyroid Carcinoma using SPECT/CT Imaging Analysis" นางสาวณัฐชยา รตะสุขารมย์, นายวสุรัตน์ ขำภาษี, นางสาวเสาวนีย์ จิตรศิลป์ฉายากุล, อาจารย์ ดร.กิติวัฒน์ คำวัน, อาจารย์ ดร.ธีระพล สลีวงศ์ และ รองศาสตราจารย์ ดร.ศักนัน พงศ์พันธุ์ผู้ภักดี มหาวิทยาลัยเทคโนโลยีพระจอมเกล้าธนบุรี และ จุฬาลงกรณ์มหาวิทยาลัย

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HOW WE TEACH | *Classroom and Laboratory Research Projects*

Interactive laboratory classes enhance neurophysiological knowledge in Thai medical students

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Wongjarupong N, Niyomnaitham D, Vilaisaktipakorn P, Suksiriworaboot T, Qureshi SP, Bongsebandhu-phubhakdi S. Interactive laboratory classes enhance neurophysiological knowledge in Thai medical students. *Adv Physiol Educ* 42: 140–145, 2018; doi: 10.1152/advan.00046.2017.—Interactive laboratory class (ILC) is a two-way communication teaching method that encourages students to correlate laboratory findings with materials from lectures. In Thai medical education, active learning methods are uncommon. This paper aims to establish *1*) if ILCs would effectively promote physiology learning; *2*) if effectiveness would be found in both previously academically high-performing and low-performing students; and *3*) the acceptability of ILCs to Thai medical students as a novel learning method. Two hundred seventy-eight second-year medical students were recruited to this study. We conducted three ILC sessions, which followed corresponding lectures. We carried out multiple-choice pre- and post-ILC assessments of knowledge and compared by repeated-measures ANOVA and unpaired *t*-test. Subgroup analysis was performed to compare high-performance (HighP) and low-performance (LowP) students. After the ILCs, participants self-rated their knowledge and satisfaction. Post-ILC test scores increased significantly compared with pre-ILC test scores in all three sessions. Mean scores of each post-ILC test increased significantly from pre-ILC test in both LowP and HighP groups. More students self-reported a "very high" and "high" level of knowledge after ILCs. Most students agreed that ILCs provided more discussion opportunity, motivated their learning, and made lessons more enjoyable. As an adjunct to lectures, ILCs can enhance knowledge in medical students, regardless of previous academic performance. Students perceived ILC as useful and acceptable. This study supports the active learning methods in physiology education, regardless of cultural context.

active learning; interactive; medical education; neurology; physiology

INTRODUCTION

Physiology educators now tend to utilize active learning strategies to help students understand complex material better in a short time (4, 5, 11, 14, 15). Active learning methods describe those that encourage students to use higher thinking processes, in contrast to passive learning methods, which aim to impart core knowledge with little interaction between the student and teacher (2, 7, 16). In medical education, the integration of clinical sciences and basic sciences in preclinical years enhances the application of knowledge to patient care in the clinical years and postgraduate work (8, 13).

Interactive laboratory classes (ILCs) were introduced at Chulalongkorn Medical School to combine active and passive learning, in the context of live physiology experiments, interaction with instructors, and interaction between the students. The ILCs focused on neurophysiology, as neuroscience is one of the most complex and challenging areas of physiology. Many reports have described "neurophobia," a fear of the neurosciences due to medical students' difficulty applying their knowledge of basic sciences to clinical neurology situations (8, 9, 21). Moreover, teaching neurophysiology with interactive and active learning methods in Thailand presents a particular challenge. Traditionally, education in Thailand relied on passive methods, i.e., the teacher standing in front of the class, and the students taking notes. Thai students may be reticent, to avoid making mistakes in front of teachers and peers (18).

Given these unique challenges, this study aimed to investigate *1*) whether introducing ILC may effectively promote learning neurosciences in our student population; *2*) whether any increased effectiveness would be seen in both academically high- and low-performing students; and *3*) the acceptability of ILCs to Thai medical students as a novel learning method. We hypothesized that students would gain more knowledge of neurosciences, in both basic and clinical sciences, and that improved knowledge would be seen in both previously high-performing and low-performing students. Furthermore, we predicted that ILCs would improve students' self-rated knowledge and lead to student satisfaction.

METHODS

Ethical considerations. The study was granted ethical approval by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. All students participated voluntarily. As part of the consent process, students were informed that declining study participation would not affect their academic progress. Written consent was gathered at the time of student recruitment.

Study population. This study was carried out during a mandatory neurophysiology class in February 2016, for second-year medical students, studying at the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. From the class of 300 students in the second year, 278 students were included in the study. Twenty-two students were excluded due to incomplete attendance (Fig. 1). All students had previously completed physiology courses in respiratory, cardiovascular, gastrointestinal, urinary, and reproductive systems. Students were

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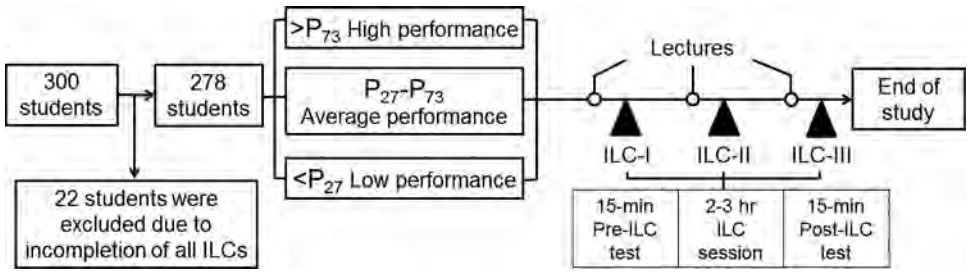

Fig. 1. Flow diagram and timeline of the study. P_{27} and P_{73}, 27th and 73rd percentile, respectively.

categorized into two groups [high-performance (HighP) and low-performance (LowP) groups], according to rankings based on collated scores from previous physiology lessons. The HighP and LowP groups were defined as students in the top 27th and bottom 27th percentile of the class, respectively. The 27th percentile was used as it represented the greatest difference in the normal distribution of population in previous literature (20).

Interventions. We conducted three neurophysiological ILCs sessions, which comprised "Physiology of Sensation" (ILC-I), "Physiology of Vision" (ILC-II), and "Physiology of Auditory and Vestibular System" (ILC-III). All students attended a 2-h lecture before each ILC. During each ILC, the instructors provided laboratory instruction, and students engaged in 3 h of interactive learning, supervised by instructors at all times. Details of the lesson content are provided in Table 1. Students were each provided with written laboratory instructions, a table for recording results, and question lists related to the experiments. Most questions were open ended with several possible answers. During the class, instructors facilitated interactive learning by encouraging the students to discuss laboratory results with each other, before asking them to explain the results based on their knowledge from the preceding lectures. Students were encouraged to generate additional questions beyond the given list. Then students presented their results and explanation of physiological mechanisms to their classmates. Before the end of each ILC, instructors summarized the key points. Pre- and post-ILC multiple-choice knowledge tests were given before and after each ILC, with each lasting 15 min.

Measurement. In this study, we compared student knowledge before and after participating in ILCs. Pre- and post-ILC tests consisted of the same questions, with a different arrangement of items and choice sequences. Questions consisted of 100% basic science-based knowledge for ILC-I, 100% clinical-based knowledge for ILC-II, and a mix of 45% basic science and 55% clinical-based knowledge for ILC-III. Value-added percentages were calculated from post-ILC percentage score subtracted pre-ILC percentage score.

Student self-evaluation and satisfaction. After each ILC, students completed self-evaluation and satisfaction forms. For the self-evaluation form, students scored their perceived level of knowledge before and after the ILCs (grading as very high, high, moderate, low, and very low). The satisfaction evaluation asked students to rate their perceptions of ILC improving their learning motivation and increase in learning pleasure in each class. Scores were graded into strongly agree, agree, moderate agree, disagree, and strongly disagree.

Statistical analysis. Baseline demographic data and student feedback were analyzed descriptively. We used repeated-measures analysis of variance (ANOVA) and unpaired *t*-test to determine statistical differences between pre- and post-ILC tests of each class and among the three classes of ILCs. The correlation of self-evaluation and summation of value-added percentages were applied by χ^2 test. The accepted level of significance was $P < 0.05$. All data were presented as means ± SD. Statistical analyses were performed using IBM SPSS Statistics version 23.

Table 1. *Contents of each interactive laboratory class and examples*

ILC	Contents of Laboratory Class	Examples of Interactive Activities
ILC-I: Physiology of Sensation	• Skin sensation, including temperature, fine touch, and pain sensation • Olfactory sensation of various odorants • Taste sensation of various substances	• Explanation of greater density of pain spots than touch spots, and greater density of cold spots than hot spots in the same skin area • Perform two-point discrimination in various areas of body and explain why they are not equal • Explanation of why subjects smell of tincture iodine less after long period of tincture iodine exposure • Explanation of why reduced tissue perfusion creates allodynia
ILC-II: Physiology of Vision	• Accommodation and light reflex examination • Visual acuity, visual field, and color vision test • Retinal examination with ophthalmoscope	• Discussion of pathway of accommodation and light reflex along with possible abnormal findings and examples of diseases • Discussion of interpreting results from visual acuity, visual field, and color vision test • Ophthalmoscopy practice
ILC-III: Physiology of Auditory and Vestibular System	• Ear examination with otoscope • Audiometric testing and interpretation • Weber's and Rinne's test • Nystagmus (triggered by rotating chair)	• Audiometry and otoscope practice • Discussion of interpretation of otoscope findings and audiometric testing • Explanation of how to distinguish conductive hearing loss from sensorineural hearing loss with Weber's and Rinne's test • Description of mechanism of nystagmus and its pathway
All ILCs	• Discuss and summarize contents at the end of each class	• Evaluate and give feedback to each student

ILC, interactive laboratory class.

Table 2. *Student characteristics*

Age, yr	All Students	Low-Performance Students	High-Performance Students
n	278	75	75
19	1 (0.4)	0 (0.0)	1 (1.3)
20	131 (47.1)	30 (40.0)	35 (46.7)
21	128 (46.0)	38 (50.7)	33 (44.0)
22	16 (5.8)	6 (8.0)	5 (6.7)
23	1 (0.4)	1 (1.3)	0 (0.0)
28	1 (0.4)	0 (0.0)	1 (1.3)
Men	151 (54.3)	48 (64.0)	41 (54.7)

Values are *n*, no. of students (with percentages in parentheses).

RESULTS

Participant characteristics. The study recruited 278 participants, of which 151 (54.3%) were men. The median age was 21 yr, with a range of 19–28 yr (Table 2). In subgroup analysis, 75 students with the lowest scores and 75 students with the highest scores according to exam scores from previous physiology lessons were assigned as the LowP and HighP group, respectively. In the LowP group, 64.0% were men, with a median age of 21 yr, whereas in the HighP group, 54.7% were men, with a median age of 21 yr.

Overall scores of pre-ILC and post-ILC tests. Post-ILC scores were significantly higher than pre-ILC scores in all ILCs (Fig. 2). In ILC-I [15 multiple-choice questions (MCQs)], the mean score increased from 9.44 ± 1.48 (62.90 ± 9.88%) to 13.90 ± 0.91 (92.64 ± 6.05%); $P < 0.001$. In ILC-II (12 MCQs), the mean score increased from 6.24 ± 1.84 (52.00 ± 15.31%) to 10.72 ± 1.23 (89.32 ± 10.21%); $P < 0.001$. In ILC-III (11 MCQs), the mean score increased from 5.46 ± 1.70 (49.60 ± 15.50%) to 8.52 ± 1.50 (77.47 ± 13.55%); $P < 0.001$. Overall, students had significant improvement in post-ILC scores compared with pre-ILC scores in all sessions (repeated-measures ANOVA, $P < 0.001$).

In subgroup analysis, the mean scores of each post-ILC test significantly increased from pre-ILC test in both LowP and HighP groups (repeated-measures ANOVA, $P < 0.05$ and $P < 0.05$, respectively). The respective mean scores of the LowP group in pre-ILC and post-ILC tests were 61.51 ± 11.15 and 90.67 ± 6.20% in ILC-I, 50.56 ± 14.65 and 87.89 ± 9.61% in ILC-II, and 48.00 ± 16.41 and 73.45 ± 15.87% in ILC-III, respectively. The mean scores of the HighP group in pre-ILC and post-ILC tests were 66.40 ± 8.66 and 93.78 ± 5.94% in ILC-I, 56.56 ± 14.52 and 91.67 ± 10.86% in ILC-II, and 53.94 ± 14.90 and 81.33 ± 11.57% in ILC-III, respectively. The average scores of pre-ILC and post-ILC tests from all sessions were 53.36 ± 8.81 and 84.00 ± 7.24% in the LowP group and 58.96 ± 7.18 and 88.93 ± 5.94% in the HighP group, respectively. Scores of the HighP group were significantly higher than those of the LowP group in all pre-ILC tests (unpaired *t*-test, $P = 0.003$, 0.01, and 0.02 in ILC-I, II, and III, respectively) and post-ILC tests (unpaired *t*-test, $P = 0.02$, 0.02, and 0.001 in ILC-I, II, and III, respectively) (Fig. 3).

Additionally, we analyzed data for the students in the LowP and HighP groups using their pre-ILC scores and post-ILC scores within each group. In the pre-ILC test scores for 75 LowP students, there were 27 (36%) high-performing (higher 27th percentile), 42 (56%) average-performing, and 6 (8%) low-performing (lower 27th percentile). In the post-ILC scores for the LowP group, there were 55 (73%) high-performing, 18 (24%) average-performing, and 2 (3%) low-performing students. For the 75 students in the HighP group, based on the pre-ILC score there were 16 (21%) high-performing, 36 (48%) average-performing, and 23 (31%) low-performing students. The post-ILC scores for the HighP students found that 32 (47%) were high-performing, 33 (44%) average-performing, and 10 (13%) low-performing students.

The value-added percentages of the LowP group were higher than those of the HighP group in ILC-I and ILC-II (29.16 ± 11.43 and 27.38 ± 9.53% in ILC-I, 37.33 ± 15.40 and 35.11 ± 15.76% in ILC-II, respectively). Conversely, the value-added percentage of the LowP group was lower than that

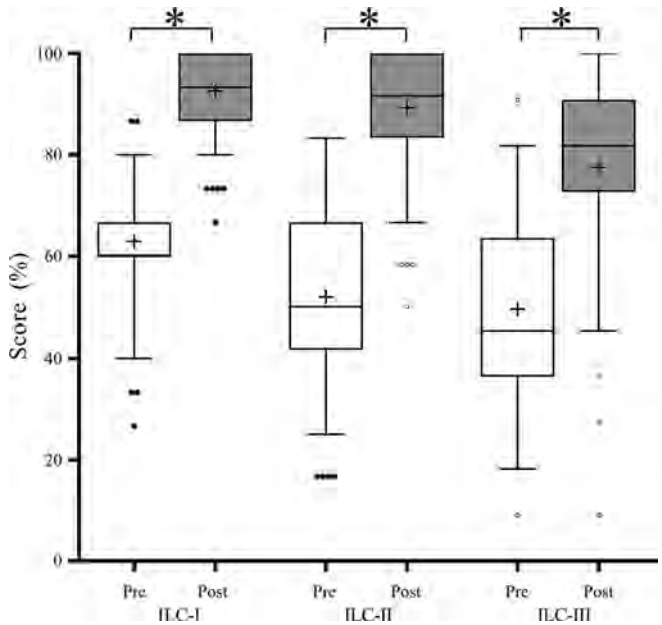

Fig. 2. Box and whisker plots (2.5–97.5 percentile) showing distribution of pre- and post-ILC assessment scores of all students in ILC-I, -II, and -III. *Significantly different ($P < 0.05$).

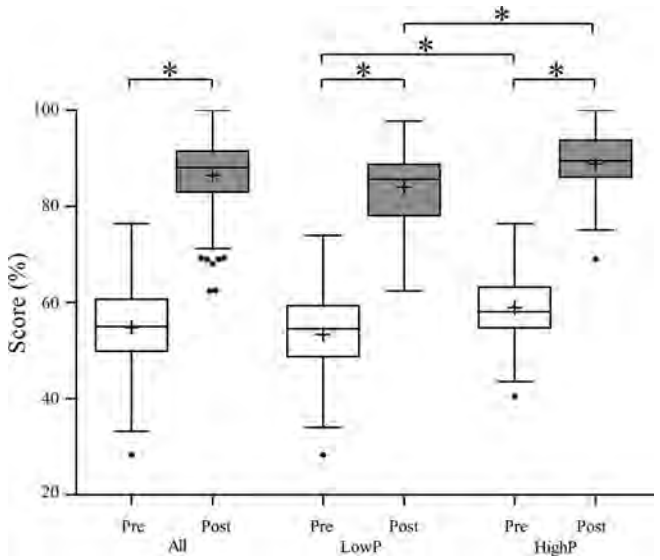

Fig. 3. Box and whisker plots (2.5–97.5 percentile) showing distribution of pre- and post-ILC test score summation of all, low-performance (LowP), and high-performance (HighP) students. *Significantly different ($P < 0.05$).

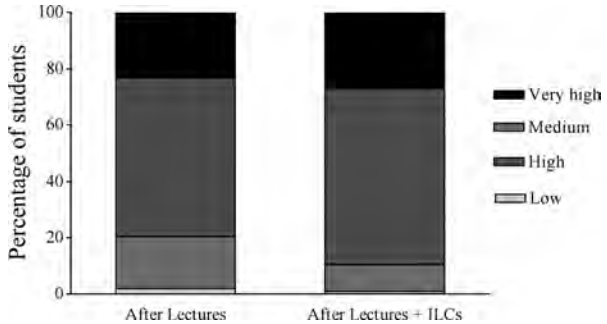

Fig. 4. 100% Stacked column chart of student self-evaluations of their knowledge before and after ILCs. Note: no students chose the "very low" option.

of the HighP group in ILC-III (25.45 ± 19.83 and 27.39 ± 16.61%, respectively). However, there was no statistically significant value-added percentage between LowP and HighP groups (unpaired *t*-test, $P > 0.05$ in all sessions, and repeated-measures ANOVA, $P = 0.44$).

Student self-evaluation and satisfaction. Evaluation forms were completed by 211 participants. Of these participants, there were 49 (23.2%) and 119 (56.4%) students, respectively, who rated their knowledge as "very high" and "high" before ILCs, and following ILCs there were 57 (27.0%) and 132 (62.6%) students, respectively, who chose "very high" and "high" to rate their level of knowledge. There were 39 (18.5%) and 4 (1.9%) students, respectively, who rated their knowledge as "moderate" and "low" before ILCs, whereas there were 20 (9.5%) and 2 (0.9%) students, respectively, who chose "moderate" and "low" following the ILCs. No students chose "very low" either before or after ILCs (Fig. 4). In addition, most students chose "strongly agree" and "agree" that ILCs, combined with lectures, gave higher learning motivation, more opportunities for discussion, and were more enjoyable comparing to lectures alone (Table 3).

DISCUSSION

This study demonstrated increased neurophysiology knowledge following ILCs, as the mean scores of the post-ILC tests were significantly higher than those of the pre-ILC tests in all three sessions. Furthermore, ILCs improved self-evaluated knowledge and led to learning satisfaction in medical students.

Potential means by which ILCs enhance learning should be considered. First, in physiology teaching, interactive methods that enhance discussion between students and instructors is beneficial (12, 17). ILCs emphasize interactive communication among students and between students and instructors. Students have opportunities to learn from each other and to ask their instructors for help. Instructors can monitor students in the midst of experiments and give advice immediately. Second, ILCs are "learning by doing" classes. During ILCs, complex ideas are demonstrated through basic science-based experiments and clinical-based investigation, allowing students to develop their physiology knowledge more than in a traditional lecture alone. In addition, we provided several sets of challenging open-ended questions during ILCs and asked students to propose their own questions. This encouraged students to think and challenge themselves beyond the traditional classroom setting.

Active learning methods, such as problem-based learning and team-based learning, have been widely applied throughout the medical curricula (1, 6, 10, 19). However, these methods demand that students study independently and search out materials. The ILC is an active learning intervention that does not have these disadvantages.

In the subgroup analysis, the HighP group scored more highly than the LowP group in both pre- and post-ILC tests in all ILCs. One might anticipate that the HighP group would have more proficient learning skills and should have a greater score improvement compared with the LowP group. However, the results showed that both the HighP and LowP groups improved their knowledge after experiencing ILCs, and the value added between the two groups was not significantly different. Our results suggest that ILCs are effective for student learning, despite previous academic performance. This finding is correlated with a previous study from Anwar et al. (1), who showed that active learning could increase the number of high-performing students compared with didactic lecture.

There were notable differences between the three ILCs. Each contained subject material in different proportions, with 100% basic science in ILC-I, 100% clinical-based knowledge in ILC-II, and mixed (45% basic science and 55% clinical-based material) in ILC-III. All ILCs led to improved knowledge, as indicated by value-added percentages following post-ILC tests. However, ILC-II, which was purely composed of clinical-based materials, led to the most improved test score. This is similar to findings from Boshuizen et al. (3) and Jozefowicz (9), who defined the term "neurophobia," and suggests that the greatest benefit of interactive class is aiding the students transition from theoretical to clinical understanding of physiology.

Interestingly, before the ILCs, ~79% of the students reported that they had a high level of knowledge of the material, but the mean scores on the knowledge tests were only 62, 52, and 50% in ILC-I, ILC-II, and ILC-III, respectively. After the ILC, students' self-assessments of their knowledge were more accurate, with 89% reporting high knowledge, and mean scores on the knowledge tests of 92, 89, and 77%, for ILCs-I, -II, and -III, respectively. In addition, student evaluation of ILCs revealed that the students found the ILCs effective and enjoyable. Most students responded with "agree" and "strongly agree" that ILC classes motivated learning more than lectures. Previous literature has reported active learning classes are

Table 3. *Student ILC evaluations*

	Strongly Agree	Agree	Not Sure	Disagree	Strongly Disagree
ILCs motivated learning more than lectures.	73 (34.60)	83 (39.34)	47 (22.27)	8 (3.79)	0 (0.00)
ILCs gave more opportunities to discuss and give opinions.	74 (35.07)	96 (45.50)	38 (18.01)	3 (1.42)	0 (0.00)
ILCs were enjoyable.	79 (37.44)	95 (45.02)	32 (15.17)	4 (1.90)	1 (0.47)

Values are *n*, no. of students (with percentages in parentheses). $n = 211$ Total students responding. ILC, interactive laboratory class.

considered more enjoyable than conventional lectures (4). However, our study has demonstrated similar findings in a Thai medical student population, where interactive learning is more unusual, further supporting the effectiveness of ILCs as a useful and acceptable learning activity, regardless of cultural context.

Introduction of ILCs requires consideration of certain trade-offs. To facilitate the sessions, a large number of faculty members are required (at least 1 facilitator to 20 students). Furthermore, as students engage with activities at different speeds, some finish work at different times, which provides a challenge for planning and results in some students waiting for their peers to finish. These difficulties are not unexpected in interactive teaching and should be weighed against the benefit to student learning. In our case, we took this time management issue as an opportunity to encourage students to teach other in their group while waiting. In addition, we provided an additional set of challenging questions for those who finished early.

Our study had some limitations. There was no control group in this study. Given the practicalities of organizing the medical curriculum, it was not feasible to introduce this new teaching activity for some of the students and not others. In addition, the postteaching assessment was administered immediately after each ILC. The results may pertain only to short-term memory and not long-term learning. To explore long-term effects of this intervention, future study is warranted.

Further study. Further studies to investigate long-term effects of ILCs, including sustained effects on knowledge level and retention, performance in future assessments, and effects on medical practice are suggested. We aim to enhance our evidence through performing an experimental study with a comparison group. Moreover, the self-evaluation of student knowledge in the present study was acquired as an overall evaluation for all ILCs. Individual assessment for each ILC would be beneficial, to correlate the evaluation with the improved score. In addition, it will be fruitful to assess the actual change in satisfaction following ILCs, i.e., measuring satisfaction level following lecture alone, before ILC, and then again after the ILC. Future study should also consider whether ILCs, or similar interactive, active learning methods may be applied to other parts of our medical curriculum.

Conclusion. ILCs are an effective interactive learning method to enhance basic science and clinical knowledge of physiology among medical students. In terms of objective assessment, we found that student neurophysiology knowledge increased after attending ILCs, regardless of previous academic performance. Subjectively, the majority of students perceived that their level of knowledge was "high" and "very high" after ILCs and were satisfied with the learning method. This is particularly noteworthy, as active learning methods are not commonplace in Thai medical education, suggesting ILCs may be effective in various cultural contexts. We support introducing ILCs into medical curricula as an adjunct to traditional lectures to augment student learning in physiology.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

N.W., D.N., P.V., T.S., and S.B.-p. conceived and designed research; N.W., D.N., P.V., T.S., and S.B.-p. performed experiments; N.W. and P.V. analyzed data; N.W., P.V., S.P.Q., and S.B.-p. interpreted results of experiments; N.W. prepared figures; N.W., D.N., P.V., and T.S. drafted manuscript; N.W., P.V., T.S., S.P.Q., and S.B.-p. edited and revised manuscript; N.W., D.N., P.V., T.S., S.P.Q., and S.B.-p. approved final version of manuscript.

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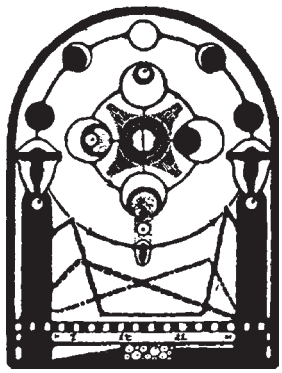

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HOW WE TEACH | *Curricular Integration of Physiology*

Attitude, but not self-evaluated knowledge, correlates with academic performance in physiology in Thai medical students

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Kwankajonwong N, Ongprakobkul C, Qureshi SP, Watanatada P, Thanprasertsuk S, Bongsebandhu-phubhakdi S. Attitude, but not self-evaluated knowledge, correlates with academic performance in physiology in Thai medical students. *Adv Physiol Educ* 43: AQ: 2 000 – 000, 2019; doi:10.1152/advan.00047.2019.—Positive attitude AQ: 3 and self-evaluation are necessary for medical students and doctors. To explore how best to integrate physiology teaching in our Thai medical curriculum, we investigated relationships between student's academic performance, their attitude, self-evaluated knowledge, and proportion of physiology taught in an organ-system integrated block. We organized 13 physiology laboratory classes, during which students self-rated attitude and knowledge. Academic performance was measured by formative and summative assessments. One hundred thirty-six participants were categorized into most proactive (Most PA), more proactive (More PA), less proactive (Less PA), and least proactive (Least PA) attitude groups by self-preparation questionnaire. Eighty participants were categorized into high (HighE), moderate (ModerateE), and low (LowE) self-evaluation rating groups. Mean formatives score in the Most PA group was significantly higher than in the other PA groups ($P = 0.003$, $P = 0.001$, and $P < 0.001$, respectively). Mean summative score in the Most PA group was significantly higher than the Less PA and the Least PA groups ($P = 0.017$ and $P = 0.015$ respectively). There was no significant difference in mean assessment scores among HighE, ModerateE, and LowE groups. Proportion of teaching time dedicated to physiology positively correlated with student attitude ($r = 0.84$, $P = 0.001$) and negatively correlated with self-evaluation rating ($r = -0.73$, $P = 0.007$). Thai medical students may benefit from a proactive attitude to studying physiology, contrasting with traditional didactic expectations of Thai education. Proportion of teaching time dedicated to physiology does not influence academic performance; therefore, future adjustments to curriculum integration may incorporate classes that facilitate self-directed learning. Future study should explore other influences on learning and assessment performance.

assessment; attitude; curriculum integration of physiology; physiology education; self-evaluation

INTRODUCTION

Success of medical students' learning and academic performance is largely measured by their performance in assessments. Many previous studies have investigated potential factors influencing academic performance (1, 15, 16). Students' attitude has been recognized as one such factor in university students (6). Previous studies in medical students have also established that students with more positive attitudes have better academic performance (19, 22). For example, previous authors have indicated a more positive attitude as corresponding to students studying after class (22). However, medical students' attitude appears to become more negative as they progress through their studies. Although reasons for the change in attitude are not clear, the negative effect of the medical curriculum is involved (22).

As medical knowledge is continuously expanding, self-evaluation of one's own knowledge limitations and learning needs is considered to be a crucial attribute for life-long professional learning among medical doctors (9). However, the ability to self-evaluate is not easily acquired. Previous studies of medical students have found low predictability of self-evaluation of academic performance (18). The students' estimates of their performance were not accurate compared with their actual performance. Many factors, such as sex, anxiety, and integrated block curricula, are found to be associated with the accuracy of self-evaluation (3–5, 11).

Contemporary universities adopt medical curricula that are systems based, with integrated teaching blocks for organ systems. These differ from traditional discipline-based curricula and are designed to facilitate students in relating knowledge of physiology, anatomy, biochemistry, and other disciplines to solve problems related to each organ system (21). The integrated curriculum is intended to reduce the sense of fragmentation and information overload of individual disciplines (7, 10). Students receiving integrated teaching may perform better academically than those receiving traditional teaching (7, 12, 14). Conversely, one challenge presented by integrated curricula is discerning how much time should be allocated for physiology, since physiology may appear complicated for students in their early years of medical education. Students misunderstanding the scope of what they should learn are at risk of superficial learning. It has been speculated that students may form negative attitudes toward learning physiology principles, as their importance may be underestimated in integrated curricula (11).

In our setting of a Thai medical school with an integrated medical curriculum, we are seeking to maximize physiology learning. It is, therefore, essential to consider the factors that influence academic performance of our medical students in AQ: 1

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physiology to inform how we integrate and deliver physiology teaching in our curriculum and in our cultural context.

Study aims. This study continues to build on a body of research in which we investigate how medical students in our Southeast Asian context learn and perform in physiology and how best to integrate and deliver physiology teaching into the undergraduate medical curricula in Thailand (23). Medical education in Thailand tends to rely on more didactic, teacher-led teaching and learning activities compared with self-directed and student-centered learning, which would be expected in Western contexts. As much existing medical education literature is based in Western cultural contexts, we seek to produce a body of evidence that contributes to curriculum integration and teaching of physiology that is relevant in the context of Thai and other Southeast Asian medical students.

This study aimed to investigate the factors that influence academic performance in physiology for Thai medical students. Based on background literature, we identified proactive student attitude and student self-evaluated knowledge as useful factors to study, and we wished to know how these may affect our student population. The specific aims we sought to address were as follows. The first is to investigate the relationship between students' attitude and academic performance. The second is to investigate the relationship between students' self-evaluated knowledge and academic performance. The third is to investigate any relationship between students' attitude and self-evaluated knowledge. The fourth is to investigate whether the proportion of teaching time dedicated to physiology in the integrated medical curriculum influences students' attitude, students' self-evaluated knowledge, and student's academic performance.

We hypothesized that academic performance would be positively associated with both positive students' attitude and with students' self-evaluated knowledge. We also hypothesized that students' attitude would be positively associated with self-evaluated knowledge. In addition, we hypothesized that the proportion of teaching time would be positively associated with students' attitude, self-evaluated knowledge, and academic performance.

METHODS

Ethical considerations. The study was granted research ethics approval by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. All students participated voluntarily. In the process of recruitment, students were informed that declining to participate would not affect their grades. Informed consent was obtained in writing from all participants.

Study population. Medical training in Thailand requires completion of a 6-yr undergraduate medical degree before being qualified to begin practice as a Doctor of Medicine (MD). Thai undergraduate medical curricula are typically divided into a 3-yr preclinical phase and 3-yr clinical phase. This study was carried out in medical students during their second (preclinical) year from June 2016 to February 2017, in the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. In total, 298 students in the class were invited to participate in the study. The current second-year curriculum was organized into organ system integrated blocks resulting from the collaboration of instructors from numerous departments. Therefore, physiology laboratory classes (LCs) were distributed throughout the year according to the correlating blocks.

Interventions. Throughout the second year of the medical degree program, we organized 13 physiology LC sessions, the details of which are presented in Table 1. All students were advised to read the laboratory directions for preparation a few days before each LC. Whether individual students reviewed materials in the advance of LCs was used as a proxy marker of their attitude, i.e., whether proactive or not. During each LC session, the instructors introduced the student to relevant physiological material and provided instruction. Then the students engaged in practicing examination skills or interpreting and discussing laboratory data. Before the end of each LC, the instructors summarized essential content, and the students undertook a formative assessment.

At the end of the formative assessments, the participants were asked whether they had prepared for the LCs in advance, for example, "Did you read the laboratory directions before this LC?" The answers were used to categorize participants based on "attitude rating." Affirmative answers were counted as positive responses.

Participants were also asked if they satisfactorily understood content of the LC, for example, "Do you understand gastrointestinal secretion more thoroughly after this LC?" The answers were used to categorize them based on "self-evaluation rating." Responses indicating understanding were counted as positive responses. The percentage of the participants who had positive and negative responses to these questions in each individual LC was calculated.

For an overall view of attitude throughout the second year of the medical degree, we categorized students by 13 attitude questions from all 13 LCs. Students who had positive responses to the attitude question more than five times, four to five times, two to three times, and zero to one time were assigned as most proactive attitude (Most PA), more proactive attitude (More PA), less proactive attitude (Less PA), and least proactive attitude (Least PA) groups, respectively. Separately, students were categorized by 13 self-evaluation questions from all 13 LCs. Participants who had positive responses to the self-evaluation question >10 times, 9–10 times, and <9 times were assigned as high (HighE), moderate (ModerateE), and low self-evaluation rating groups (LowE), respectively.

Although physiology LCs occur as part of organ system integrated blocks, the weight of, or focus on, the physiology teaching does not equally distribute across each block. Thus we defined "proportion of physiology" as proportion of the physiology teaching time to total teaching time in that individual block, to determine how much physiology contributes to the teaching of that organ system in our degree. For example, LC-8: Physiology of Gastrointestinal Secretion, was in the gastrointestinal system block. The total time of every lecture, laboratory, and case discussion in the block was 50 h. Because there was 18 h contributed to physiology out of 50 h, we calculated proportion of physiology for LC-8 by 18 h divided by 50 h, which equates to 36%.

Outcome measurement. The outcome measurements were obtained as formative and summative assessment scores. The formative physiology assessments were conducted ~15 min after each LC. The assessments consisted mostly of multiple-choice questions, with some true/false questions, and examined basic and clinical physiological knowledge. This score was intended for early assessment of learning outcomes and did not contribute to summative grades. After the end of each block, the students undertook the integrated summative assessment. Summative assessments were integrated and consisted of multiple-choice questions covering all disciplines in the blocks, e.g., physiology, anatomy, biochemistry, etc. The summative scores were used to assess learning corresponding to long-term learning outcomes. The formative assessment was used for early assessment of the learning outcomes, whereas the summative assessment was used to evaluate long-term learning. Both formative and summative assessments were carefully designed to align with the curricular learning outcomes and the teaching and learning activities carried out in LCs, correlating with the concept of constructive alignment (2).

Statistical analysis. Demographic data were analyzed descriptively and were recorded for every participant who completed any

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Table 1. *Contents of each laboratory class and its details*

LC	Contents of LCs	Format of LCs	Organ System Block of LC in the Medical Course	Physiology Proportion of Total Teaching Time, %
LC-1: Autonomic Nervous System Integration	• Demonstration of effects of autonomic nervous system drugs in dog	Interpreting laboratory data	Fundamentals of Tissue Biology and Human Function	29.31
LC-2: Physiology of Nerve and Neuromuscular Junction	• Demonstration of effects of neuromuscular-blocking drugs on muscle contraction in frog	Interpreting laboratory data	Musculoskeletal System	8.24
LC-3: Physiology of Skeletal Muscle	• Demonstration of contraction periods and summation of contraction of muscle in frog	Interpreting laboratory data	Musculoskeletal System	8.24
LC-4: Physiology of Reflex	• Examination of superficial reflexes, deep tendon reflexes, and autonomic nervous system reflexes	Practicing examination skills	Musculoskeletal System	8.24
LC-5: Chest Movement and Breath Sound	• Physical examination of respiratory system	Practicing examination skills	Respiratory System	44.19
LC-6: Pulmonary Function Test	• Measurement of forced vital capacity and forced expiratory volume in 1 s by spirometer	Practicing examination skills	Respiratory System	44.19
LC-7: Cardiovascular System Integration	• Demonstration of cardiovascular diseases and drugs on arterial pressure and heart rate in dog	Interpreting laboratory data	Cardiovascular System	72.14
LC-8: Physiology of Gastrointestinal Secretion	• Demonstration of salivary, bile, and pancreatic secretions in dog	Interpreting laboratory data	Gastrointestinal System	36.00
LC-9: Renal Function	• Demonstration of effects of hemorrhage, drugs, and some substances on renal function in dog	Interpreting laboratory data	Urinary System	45.61
LC-10: Reproductive Physiology	• Demonstration of effects of male and female sex hormones on estrous cycle, secondary sex organs, body weight, and activity of rats	Interpreting laboratory data	Reproductive System	55.17
LC-11: Physiology of Sensation	• Examination of skin sensation, olfactory sensation of various odorants, and taste sensation of various substances	Practicing examination skills	Neuroscience	30.49
LC-12: Physiology of Vision	• Accommodation and light reflex examination • Visual acuity, visual field, and color vision test • Retinal examination with ophthalmoscope	Practicing examination skills	Neuroscience	30.49
LC-13: Physiology of Auditory and Vestibular System	• Ear examination with otoscope • Audiometric testing and interpretation • Weber's and Rinne's test • Nystagmus (triggered by rotating chair)	Practicing examination skills	Neuroscience	30.49

LC. laboratory class.

number of questionnaires. Repeated-measures analysis of variance (ANOVA) was used to analyze the difference of total formative and summative scores among different student groups categorized by overall attitude and self-evaluating questions from all LCs. Fisher's least significant difference was used as post hoc analysis to explore differences between pairs of mean scores of different student groups. Only participants who completed all questionnaires for attitude and for self-evaluation were included in the respective final analysis for each category. Additionally, students' attitude and self-evaluation rating were analyzed for possible relationships to the proportion of physiology. This analysis was done by using bivariate correlation (Pearson's correlation coefficient, r). The accepted level of significance was set at $P < 0.05$. The averaged formative and summative scores are presented as means ± SD. Statistical analyses were performed using IBM SPSS Statistics version 23.

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RESULTS

Participant characteristics. Of the recruited 298 participants, 161 (54.0%) were men and 137 (46.0%) were women. Because some students did not respond to attitude questions or self-evaluation questions in some LCs, there were only 136 participants (45.6%) who completed all attitude questions. Categorizing by attitude questions, 10 participants (7.4%) were classified in the Most PA group, 22 (16.2%) in the More PA group, 62 (45.6%) in the Less PA group, and 42 (30.9%) in the Least PA group. There were 80 participants (26.8%) who completed all of the self-evaluation questions. Categorizing by the self-evaluation questions, 50 participants (62.5%) were classified in the HighE group, 22 (27.5%) in the ModerateE, and 8 (10%) in the LowE group.

Relationship between assessment scores and attitude. The total number of participants included in this analysis was 136. The mean scores of the formative assessments from all sessions were 80.76 ± 6.72 in Most PA, 73.40 ± 5.94 in More PA, 73.66 ± 6.52 in Less PA, and 72.70 ± 6.26 in Least PA, with a significant difference between the groups ($P = 0.005$). Post hoc analysis revealed that the mean formative score of the Most PA group was significantly higher than those of the More PA group, Less PA group, and Least PA group ($P = 0.003$, $P = 0.001$, and $P < 0.001$, respectively), whereas there was no significant difference between More PA, Less PA, and Least PA groups ($P > 0.05$) (Fig. 1*A*). F1

The mean scores of the summative assessments from all blocks were 79.90 ± 5.95 in the Most PA, 75.86 ± 6.52 in the More PA, 72.80 ± 8.97 in the Less PA, and 72.41 ± 9.55 in the Least PA group, with a significant difference between the groups ($P = 0.048$). Post hoc analysis revealed that the mean summative score of the Most PA group was significantly higher than those of the Less PA and Least PA groups ($P = 0.017$ and 0.015, respectively), but not from that of the More PA group ($P = 0.222$). There was no significant difference between the More PA, Less PA, and Least PA groups ($P > 0.05$) (Fig. 1*B*).

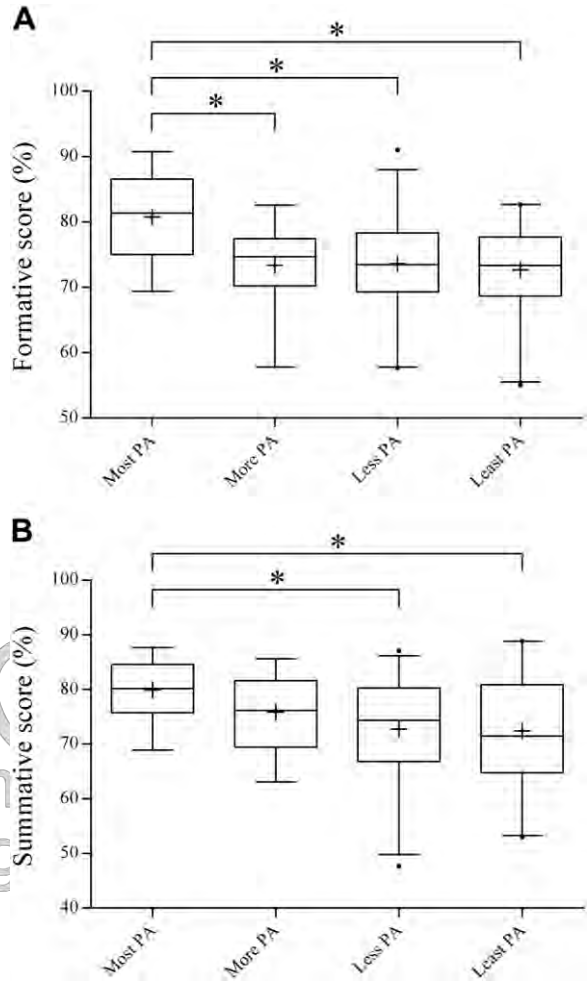

Fig. 1. Box and whisker plots (2.5–97.5th percentile) showing distribution of formative score (*A*) and summative score (*B*) of the students in the most proactive (Most PA; $n = 10$), more proactive (More PA; $n = 22$), less proactive (Less PA; $n = 62$), and least proactive (Least PA; $n = 42$) groups. The plus symbols indicate the mean scores of each group. *Significantly different ($P < 0.05$).

Relationship between assessment scores and self-evaluation. The total number of participants included in this analysis was 80. The mean scores of the formative assessments from all sessions were 75.13 ± 5.76 in the HighE, 72.39 ± 6.92 in the ModerateE, and 73.66 ± 4.72 in the LowE group. No significant difference between the groups was found ($P = 0.215$). Also, pairwise comparisons between each group did not show any significant differences ($P > 0.05$ for all comparisons) (Fig. 2*A*). F2

The mean scores of the summative assessments from all blocks were 73.86 ± 8.54 in the HighE, 70.23 ± 10.24 in the ModerateE, and 74.83 ± 11.00% in the LowE group, and no significant difference between the three groups was found ($P = 0.265$). Pairwise comparisons between each group did not show any significant differences ($P > 0.05$ for all comparisons) (Fig. 2*B*).

Relationships between proportion of physiology and students' attitude and students' self-evaluation. Students' attitude was found to have significant positive linear correlation with the proportion of physiology ($r = 0.84$, $P = 0.001$) (Fig. 3*A*), whereas the self-evaluation rating had significant negative linear correlation with the proportion of physiology ($r = -0.73$, $P = 0.007$) (Fig. 3*B*). Students' attitude did not significantly correlate with their self-evaluation rating ($r = -0.48$, $P = 0.159$) (Fig. 3*C*). F3

Relationships between proportion of physiology and assessment scores. The mean formative and summative scores of all students were calculated for each LC. No significant linear relation was found between the proportion of physiology and the mean formative score ($r = -0.30$, $P = 0.324$) (Fig. 4*A*). The proportion of physiology also did not have significant linear correlation with the mean summative score ($r = -0.39$, $P = 0.189$) (Fig. 4*B*). F4

DISCUSSION

Our analyses have led to findings with implications for the delivery of physiology teaching and assessment in Thai medical education. First, we found a proactive attitude to preparation before LCs to be associated with significantly higher academic performance in both formative and summative assessments. Preparation by reviewing materials in advance of class time may help students more effectively study physiology compared with those who attend class without preparation in

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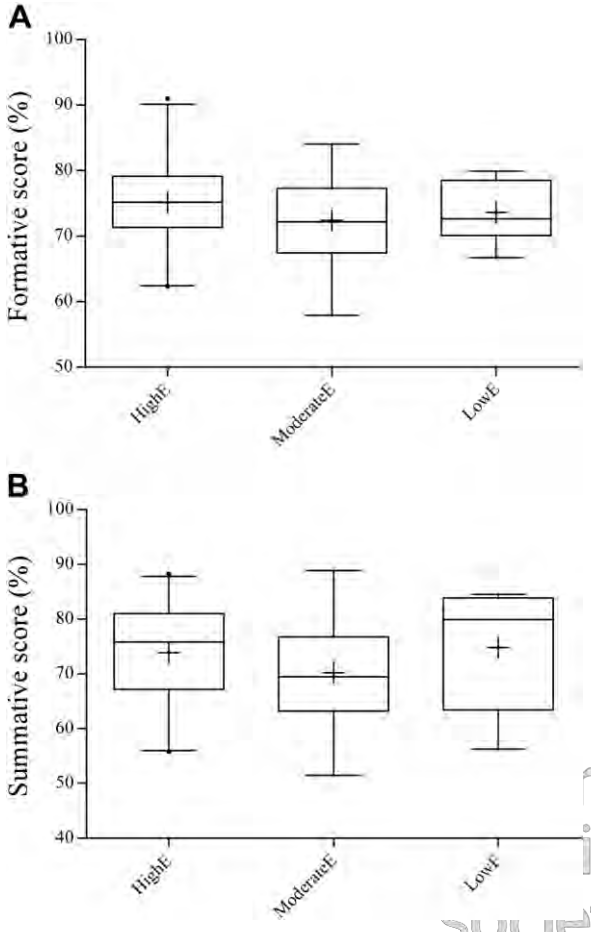

Fig. 2. Box and whisker plots (2.5–97.5th percentile) showing distribution of formative score (*A*) and summative score (*B*) of the students in the high self-evaluation (HighE; $n = 50$), moderate self-evaluation (ModerateE; $n = 22$), and low self-evaluation (LowE; $n = 8$) groups. The plus symbols indicate the mean scores of each group. No significant difference between the three groups was found.

advance and, consequently, perform better in assessments. This is noteworthy in our Thai context, where self-directed learning is less common compared with Western education. It indicates the importance of setting clear expectations for students and allows us to present evidence to our medical students that a more proactive attitude is correlated with improved assessment performance. Therefore, it is in their best interests to prepare for classes in advance.

Another key finding was that the association between attitude and formative scores was more apparent than between attitude and summative scores. One possible explanation is that a proactive attitude is short-lived and does not necessarily affect preparation for summative assessments. It is perhaps concerning that preparation a few days before LC may raise the score of the upcoming formative assessment more than the summative assessment and raises questions about what other influences may affect performance in summative assessments. This may represent a disparity between deep and superficial learning, which has been raised as a concern for integrated curricula such as ours (11). Another potential explanation is a difference in student performance between physiology-only assessments (such as in the formative assessments) and integrated assessments (such as our summative assessments). This may represent a potential reduced benefit of proactive attitude when physiology is examined in an integrated way. This raises concerns for integrated medical degrees, and potentially for clinical practice: when these students begin practice as doctors, they will need to access knowledge across scientific disciplines to solve real-world clinical problems in integrated ways.

Interestingly, we also found there was no relationship between overall self-evaluated knowledge and academic performance. For students within the HighE category (the highest

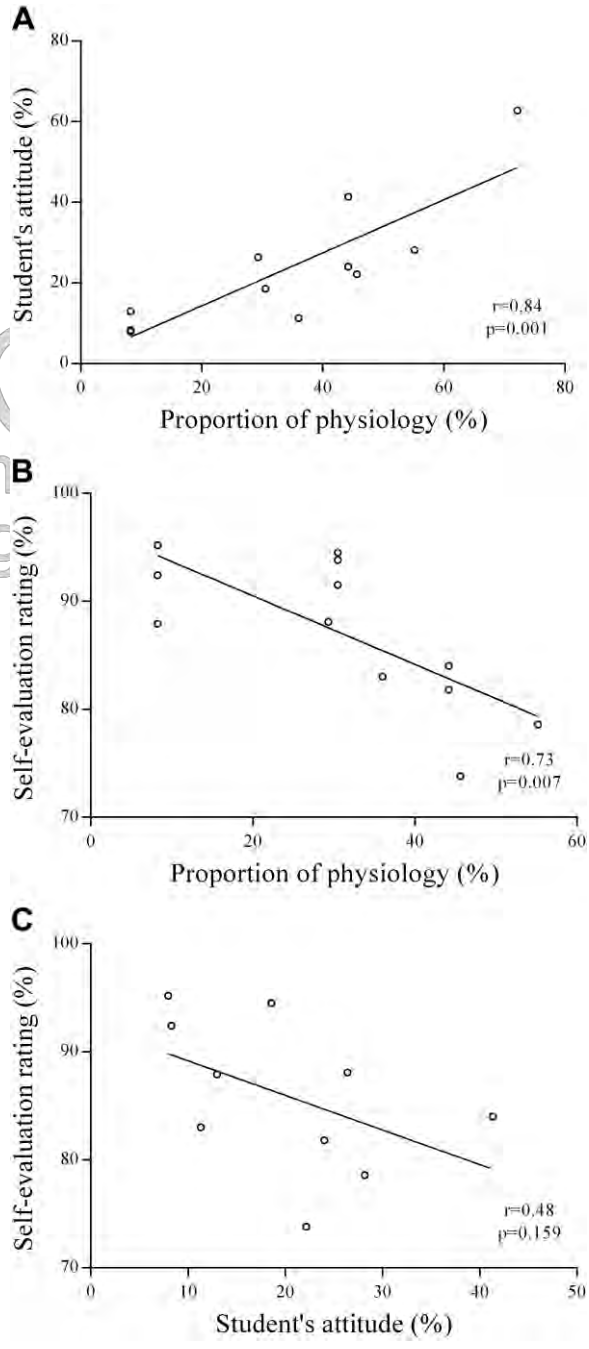

Fig. 3. Interrelationships among proportion of physiology, student's attitude, and self-evaluation rating. A strong correlation between proportion of physiology vs. students' attitude ($r = 0.84$, $P = 0.001$; *A*) and self-evaluation rating ($r = -0.73$, $P = 0.007$; *B*) was found. *C*: students' attitude has little association with self-evaluation rating ($r = -0.48$, $P = 0.159$).

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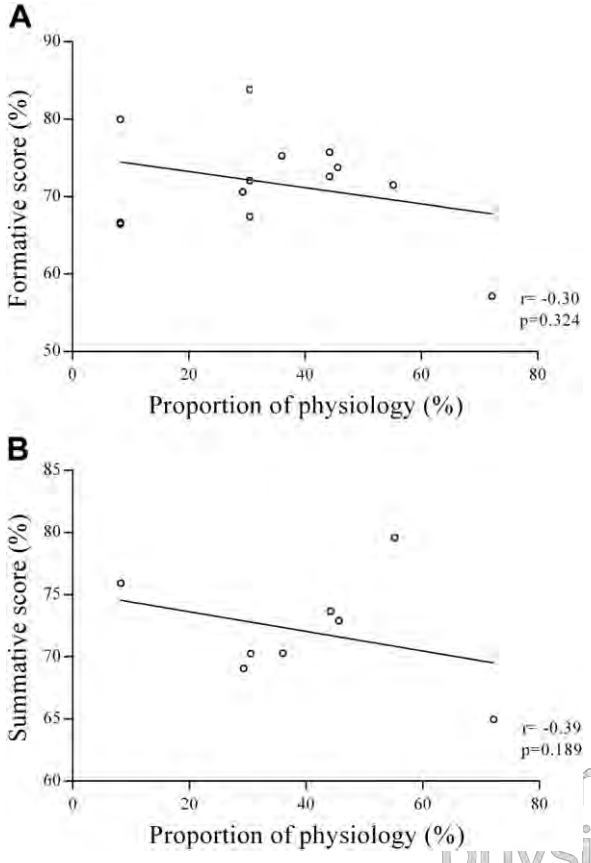

Fig. 4. Interrelationships between proportion of physiology and students' academic performance assessed with formative score (r = -0.30, P = 0.324; *A*) and summative score (r = -0.39, P = 0.189; *B*).

self-evaluated knowledge), there was a wide variation in performance on assessments. This finding was in accordance with previous findings that low-performing students tend to overestimate their academic grades (8). Low-performing students may be unable to recognize their lack of knowledge, leading to a false sense of satisfaction in one's own knowledge. Consequently, they may not be motivated to study sufficiently, compared with students who perceive their knowledge as inadequate.

The results also demonstrated that there were more students who had a positive attitude in the LCs with a higher proportion of physiology. Nonetheless, there were fewer students who gave positive responses to the self-evaluation question in the LCs with a high proportion of physiology. We can extrapolate from the findings that the greater proportion of physiology may help students gain a better recognition of their knowledge level, resulting in a reduction in self-evaluation of knowledge. Consequently, this may motivate students to study more in advance of class and lead to a more proactive attitude. An alternative view of this potential phenomenon could be explained using the Johari Window (20). Through this model, students who become aware of their own learning needs could be seen as moving from "unknown unknowns" to "known unknowns."

It is noteworthy that our findings indicate that the proportion of physiology is negatively correlated with student self-evaluated knowledge and is not directly related to higher academic performance. This raises questions of the utility of classroom time in physiology and possible alternative uses of face-to-face teaching. In some western settings, a flipped classroom format has been adopted. This involves students taking more responsibility for their own learning and studying material in their own time, while using face-to-face teaching to apply knowledge to worked examples, clear up misconceptions, and consolidate their learning from self-study (1). Although this educational approach relies on student self-directed learning, which is not usual for our cultural context, our findings imply that this approach could be effective if implemented. Conversely, the proportion of time spent on physiology teaching correlates positively with students being more proactive, and so there is a counterargument that the current format should be maintained. Therefore, the proportion of physiology may not have direct effects on academic performance, as measured by scores in both formative and summative assessments. However, the proportion of physiology may have indirect effects on academic performance through effects on students' attitude.

This study benefited from its simple, replicable design. Through this relatively simple study, we were able to follow up medical students at one university for the period of one year and derive significant insights into influences on their performance in physiology. We consider our outcomes to have been measured with valid tools, i.e., assessments that were developed by subject experts to ensure alignment with learning outcomes and teaching and learning activities. This study has made a significant contribution to existing literature by being the first to demonstrate the importance of student proactive attitude and appropriate allocation of physiology in an integrated medical curriculum in a Thai learning environment. It has led to new implications for our context related to more self-directed learning and alternative teaching approaches, such as the flipped classroom. This challenges preexisting assumptions about expectations of students and teachers in Thailand and causes us to reevaluate how we integrate physiology into our curriculum. Furthermore, our research explored the effect of proportions of physiology in organ-system integrated blocks, providing novel findings related to curriculum integration, which allows us to argue for self-directed learning in physiology among preclinical medical students in Thailand.

Limitations of our study included the small scale and the fact that the findings have not yet been replicated. Randomization was precluded due to the structure of the medical degree and because all students needed to be exposed to the same teaching. Unfortunately, there was attrition of recruited participants over the year: although all students invited to participate completed at least some of the questionnaires, we were only able to include data for those who completed the questionnaires for every LC in the respective analyses. Another potential criticism was utilizing participants' self-reporting to categorize them according to both attitude question and self-evaluation. Furthermore, for simplicity, categorization was done in a binary way, which may not capture all pertinent information. For example, participants were asked if they prepared in advance of class, but those who answered "yes" may include a spectrum of those who had only briefly reviewed, compared with others who may have spent hours studying intently. Moreover, many other factors may account for student behavior and performance in assessments other than those measured in this study. We have considered these limitations of the

present study in planning further study, as we will expanded on below.

Further study. Further study should aim to answer research questions emerging from our study: medical student proactive attitude is correlated with academic performance, yet other factors may influence behavior, learning, and academic performance. It is not clear why students have different attitudes and have different perceptions of their own physiology knowledge. Furthermore, the mechanism by which the proportion of physiology may influence academic performance and self-evaluated knowledge is unclear. The reason why a proactive attitude appears to influence formative assessment performance more than summative assessments has been speculated, but it is not certain. So far, students' attitude and self-evaluated knowledge were gathered via a binary, quantitative measurement. There are other ways in which students' attitude and factors affecting student learning and behavior may be investigated. In further study, to investigate the above outstanding questions, qualitative research should be considered, which is more useful for investigating attitudes, perceptions, and behaviors, as opposed to quantitative measurements (13). Ultimately, further study should investigate possible interventions to promote academic performance, students' attitude, along with accuracy of self-evaluation rating, potentially incorporating a flipped classroom approach and assessing the impact in our Thai context. In addition, preclinical performance of physiological knowledge in assessment is not the same as application in clinical practice. Thus future research should investigate relationships between preclinical physiology teaching, learning, and assessment, and clinical practice.

Conclusion. Proactive attitude toward physiology, demonstrated by studying in advance of LCs, was associated with higher academic performance in physiology among Thai medical students. The effect was weaker in integrated summative assessments compared with formative physiology-only assessments, raising questions of how deep, lasting learning can be encouraged, and how students prioritize studying physiology alongside other subjects in studying for integrated assessments. Negotiating disciplines in an integrated way is essential for eventual clinical practice as doctors, which will involve integrated management of patients. In contrast, self-evaluated knowledge did not predict academic performance, with a tendency for overestimation in students with low academic performance. Furthermore, a higher proportion of physiology teaching within integrated organ system blocks in the curriculum was positively correlated with students' attitude toward studying physiology, yet it negatively correlates with student's self-evaluated knowledge in physiology. There was no correlation between proportion of time spent in physiology class and performance in formative or summative assessments. Possible implications of the findings include that the traditional, didactic-based approach to teaching physiology in Thai medical education may be suboptimal, and this challenges us to consider whether more self-directed learning and alternative uses of classroom time may provide a better approach to learning physiology in an integrated curriculum. Active learning methods, such as interactive LC, have already been introduced as a new, nontraditional method for teaching in our context (23), but the findings of the present study suggest that an overall review of the integrated curriculum may be considered. However, further research is needed to explore students' learning behavior and how their beliefs and attitudes influence their performance in assessments.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors. AQ: 5

AUTHOR CONTRIBUTIONS

N.K., C.O., S.P.Q., P.W., S.T., and S.B.-P. conceived and designed research; N.K., P.W., and S.B.-P. performed experiments; N.K. and S.T. analyzed data; N.K., S.P.Q., and S.B.-P. interpreted results of experiments; N.K. and S.B.-P. prepared figures; N.K., C.O., and S.P.Q. drafted manuscript; N.K., C.O., S.P.Q., S.T., and S.B.-P. edited and revised manuscript; N.K., C.O., S.P.Q., P.W., S.T., and S.B.-P. approved final version of manuscript. AQ: 6

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Serotonin Depletion Amplifies Excitability of Trigeminal Ganglia Nociceptive Neuron: A Patch Clamp Recording Study

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Table: 1

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Authors' contribution

N.H.-a.-s., S.T. and S.B.-p. conceived and designed research;

N.H.-a.-s and S.B.-p. performed experiments;

N.H.-a.-s. and S.T. analyzed data;

N.H.-a.-s., S.T. and S.B.-p. interpreted results of experiments;

N.H.-a.-s., S.T. and S.B.-p. prepared figures;

N.H.-a.-s. and S.T. drafted manuscript;

S.T. and S.B.-p. edited and revised manuscript;

N.H.-a.-s., S.T. and S.B.-p. approved final version of manuscript.

Disclosure statement

Naeemah Haji-a-sa has nothing to disclose.

Sekh Thanprasertsuk has nothing to disclose.

Saknan Bongesebandhu-phubhakdi has nothing to disclose.

Keywords

Serotonin depletion, trigeminal ganglia, migraine, patch-clamp recording, neuronal excitability

Abstract

Serotonin (5-HT) depletion was found to be a contribution factor in migraine pathophysiology. We studied the effect of 5-HT depletion on resting membrane potential (RMP) and action potential properties of trigeminal ganglia (TG) neuron by using patch-clamp recording technique in rats. Serotonin depletion was induced by using parachloro-L-phenylalanine (PCPA). TG neurons were divided into 4 groups; small-to-medium and large size neurons from 5-HT-depleted rats (PCPA-SM and PCPA-L groups, respectively); and small-to-medium and large size neurons from control rats (control-SM and control-L groups, respectively). The RMP was found to be significantly less negative in PCPA-SM than in PCPA-L group (p=0.005). The rheobase of PCPA-L was significantly higher than PCPA-SM group p=0.004). The total spike of PCPA-SM was significantly more than PCPA-L and control-L groups (p=0.004 and 0.008, respectively). These results suggest that the application of 5-HT-depleted procedure may magnify the differences of electrophysiological properties between nociceptive and non-nociceptive TG neurons.

Introduction

Serotonin (5-HT) depletion was found to be a contribution factor in pathophysiology of migraine headache [1-3]. At present, we know that 5-HT's action on 5-HT_{1B}, 5-HT_{1D} and 5-HT_{1F} receptors of trigeminal nociceptive pathway helps modulate vascular and neuronal response [4-6]. Accumulating evidences have suggested that 5-HT depletion participates in hyperexcitability of cortical neurons, dysregulation of vascular response and facilitation of trigeminal nociceptive signaling, which potentially leads to symptoms of headache in migraineurs [1, 7-10].

Trigeminal ganglion (TG) contains the first order neuron of trigeminal nociceptive pathway, receiving pain information from nociceptors and transmitting it to the second order neuron in trigeminal nucleus caudalis (TNC). Based on a morphological study, neurons in TG were classified into nociceptive and non-nociceptive neuron according to their diameter. Small and medium diameter neurons (diameter <30 μm) are considered as nociceptive ones as they are origins of Aδ or C nerve fibers [11, 12]. They also express several different neuropeptides involving in pain signal transmission, for example substance P (SP), calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating polypeptide receptor type 1 (PACAP) [13]. While large diameter neurons (diameter ≥30 μm) are generally responsible for non-nociceptive modalities [11, 12]. Nevertheless, details regarding electrophysiological alteration of TG neuron under the 5-HT-depleted condition, as well as the difference of this alteration between nociceptive and non-nociceptive neuron, has not been well- established.

Thus, we aimed to study the electrophysiological parameters, including resting membrane potential (RMP) and action potential (AP) properties, of TG neurons in 5-HT depleted rats by using primary cell culture and whole cell patch-clamp recording technique. We also sought to determine the difference of such electrophysiological parameters between small-to-medium (SM) and large (L) size, i.e. nociceptive and non-nociceptive TG neurons. In this study, parachloro-L-phenylalanine (PCPA), the inhibitor of tryptophan hydroxylase enzyme, was the substance used for modeling 5-HT-depleted rats as in previous work [7].

Materials and Methods

Experimental Animals

Adult male Wistar rats weighing 200-300 grams purchased from Nomura Siam International, Bangkok, Thailand were use in all experiments. Rats were housed in stainless cages in the ventilated room under 12-hour

dark-light cycle and were allowed to access food and water *ad libitum*. All protocols were approved by the Animal Care and Use Committee of the Faculty of Medicine, Chulalongkorn University, Thailand (019/2561).

5-HT-depleting Procedure and Grouping

Rats were divided into 2 populations, which were 5-HT-depleted rats and controls. We injected PCPA (Sigma Aldrich, USA) (100 mg/kg body weight) into rat's peritoneal cavity for three consecutive days to induce 5-HT depletion as in previous work [7]. Based on 5-HT-depleted-procedure and neuronal diameter, rats' TG neuron would be divided into 4 groups. The first and second neuronal group were the small-to-medium sized (diameter <30 μm) and large sized (diameter ≥30 μm) neurons obtained from the 5-HT-depleted rats (PCPA-SM and PCPA-L group, respectively). While the third and fourth group were the small-to-medium sized and large sized neurons obtained from control rats (control-SM and control-L group, respectively).

TG Removal and Primary Culture of TG Neurons

TG removal and culture process were done as previously described [14-17]. Rats from both groups were sacrificed by intraperitoneal injection of thiopental (70 mg/kg body weight) following by decapitation. Both sides of TG were removed and transferred into 35-mm culture dish of ice-cold Hank's Balanced Salt Solution (HBSS) with penicillin/streptomycin (10-20 μl; 10,000 U penicillin and 10 mg streptomycin/mL). The ganglia tissue were washed in HBSS twice, were cut into small pieces using a sterile razor blade in 1 ml of HBSS and were then shifted into a plastic tube. Collagenase (100 μl; 2 mg/mL) and dispase (200 μl; 50 U/mL) enzymes were subsequently added. The sample was filtrated with 0.22 μm filter and was then incubated into water bath at temperature 37 $^{\circ}$C for 20 minutes. After that, we centrifuged the sample at RCF 400g for 1 minute to remove supernatant. The sample was then added with papain enzyme and was again incubated in 37^{o}C water bath for 20 minutes. Then we added 2 mL of L-15 medium and centrifuged the sample for 8 minutes. Next, the sample was washed with 400 μl of F-12 completed medium, placed into Laminin/PDL coated dish, and maintained in an incubator (37°C, 5% CO_2) for 3 hours. Lastly, F-12 completed medium was further changed for 2 times and the sample was maintained in an incubator for 18-24 hours before using in electrophysiological patch-clamp study.

Patch Clamp Recording

Whole-cell patch clamp recording was done using the same technique as in previous work [17]. Plastic chambers containing sample of TG neurons were set on a stand of microscope (Olympus BX51WI, Olympus, Japan). TG neurons were perfused with an external solution at a flow rate of 1 mL/min at room temperature. This external solution was composed of 145 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM D-glucose and 10% HEPES; which the pH was adjusted to 7.40 with 1 M NaOH and the osmolality was adjusted to 320±5 mOsm/kg by using D-glucose. Microelectrodes, outer diameter of 1.5 mm and an inner diameter of 0.86 mm (Sutter Instruments, Navato, CA, USA), were filled with an internal solution containing 140 mM K-gluconate, 1 mM $CaCl_2$, 10 mM EGTA, 10 mM HEPES and 10 mM ATP; which the osmolality was adjusted to 290±5 mOsm/kg with D-glucose. Patch pipettes had tip resistances between 6-8 MΩ. Neuronal diameter (μm) and morphology were noted. RMP (mV), capacitance (pF) and properties of AP including threshold (mV), rheobase (pA), AP height (mV), AP overshoot (mV), AP rising time (msec), AP falling time (msec), AP duration (msec), afterhyperpolarization (AHP) depth (mV), AHP duration (msec) and total number of spikes were recorded using an Axopatch 200B amplifier (Axon instruments, CA, USA) and clampex 10.2 software (Molecular devices, CA, USA).

Statistical Analysis

All electrophysiological parameters were summarized in mean ± standard deviation (SD). Student's t-test were performed to compare neuronal diameter between PCPA-SM and control-SM groups, and between PCPA-L and control-L groups. One-way ANOVA statistic were performed to compare the electrophysiological parameters among the four groups (IBM SPSS version 22, NY, USA). Tukey post-hoc analyses were then applied to determine intergroup differences. Statistical significance level was set at $p<0.05$.

Results

From the primary culture procedure, we obtained a total of 38 neurons, 20 from 5-HT depleted rats and 18 from control rats. Based on diameter as mentioned earlier, the TG neurons were classified into control-SM (n=10), control-L (n=8), PCPA-SM (n=9), PCPA-L (n=11) groups. Comparisons of neuronal morphology and diameter were made between PCPA-SM (figure 1A) and control-SM (figure 1C) group, and between PCPA-L (figure 1B) and control-L (figure 1D) group. There was no obvious difference in neuronal morphology. Neuronal diameter of control-SM and PCPA-SM groups were 25.13±2.79 and 24.31±3.11 μm, respectively

(p=0.56). Neuronal diameter of control-L and PCPA-L groups were 33.44±1.75 and 34.08±4.13 μm, respectively (p=0.68).

PCPA-SM group appeared to have more abundant AP development than other groups as demonstrated in the representative traces (figure 2). Electrophysiological parameters showing significant difference among the four groups were RMP, rheobase and total spikes (table 1, one-way ANOVA, p<0.05). For post-hoc analyses, the RMP was significantly more negative in PCPA-L than in PCPA-SM groups (figure 3a, post-hoc test, p=0.005). The rheobase of PCPA-L was significantly higher than PCPA-SM group (figure 3b, post-hoc test, p=0.004). The total spike of PCPA-SM was significantly more than PCPA-L and control-L groups (figure 3c, post-hoc test, p=0.004 and 0.008, respectively).

Discussion

The important findings from our study were the significant differences of the RMP, rheobase and total spikes between PCPA-SM and PCPA-L group, while such differences were not detected between control-SM and control-L group. These parameters have been used to determine neuronal excitability [17, 18]. Thus, our quantitative electrophysiological results novelly illustrated that the application of 5-HT-depleted procedure may magnify the contrast of excitability between nociceptive and non-nociceptive TG neurons. Because it appeared to promote the excitability of the small-to-medium diameter neuron, while simultaneously suppress the excitability of the large diameter one. In consistent with previous works, the 5-HT-depleted procedure participated in facilitation of trigeminal nociceptive signaling as it increased the number of Fos-immunoreactive neurons, indicator of trigeminal nociception, in TNC [7, 9].

This contrast between the nociceptive and non-nociceptive neuron may also associate with migraine headache. Evidence in human demonstrated that 5-HT depletion to be a predisposing factor of migraine attack [19]. Depletion of tryptophan, a substrate of 5-HT, was also found to be associated with headache symptom in migraineurs [20]. These can be explained by the action of 5-HT to 5-$HT_{1B/1D/1F}$ on the trigeminal nociceptive pathway, which theoretically modulates neuronal response to nociceptive stimuli [4, 5]. The suppressing effect of 5-HT depletion on the non-nociceptive part of trigeminal system, however, had not been extensively studied, especially in the field of electrophysiology.

Regardless of the remarkable differences of the parameters particularly between PCPA-SM and PCPA-L group, we did not demonstrate significant difference of any parameter neither between PCPA-SM and control-SM nor PCPA-L and control-L group. This may interpret that our 5-HT-depleted procedure is not powerful

enough to generate an absolute, i.e. nonrelative, increase in the excitability of TG neuron. It is accordingly important to denote that 5-HT depletion alone may not sufficient to cause neuronal dysfunction in migraine since pathophysiology of the disease is a complex process involving several factors [2, 3]. Despite that, the role of 5-$HT_{1B/1D/1F}$ agonists on treating headache symptom by modulating nociceptive signal transmission is unequivocal [2, 3, 21].

Our study has limitation in term of small numbers of the cultured neurons. Besides, the results did not show significant difference in any parameter other than RMP, rheobase and total spikes., which may weaken the integrity of our result to a slight extent. Additionally, we used only male rats in our work. Absence of female rats may attenuate the contrast of electrophysiological parameters among the groups, as female sex hormonal system was found to have a profound effect on triggering migraine in human and animal studies [17, 22, 23]. Recent rodent study also demonstrated the headache-liked behavioral response to CGRP to be female specific [24]. Existence of menstrual cycle in female rats, however, could lead to variation in degree of TG neuron's excitability as a result of estrogen fluctuation [17]. Thus, using only male rats should help diminish the variation of migraine susceptibility during each period of estrous cycle. Our future direction will involve on employing the female rats on a specific period of estrous cycle. Furthermore, we plan to focus in the electrophysiological patch clamp recording study of TG neuron on a more specific migraine model, such as the induction of cortical spreading depression. We also plan to do more work regarding the effect of 5-HT depletion on the non-nociceptive neuron in trigeminal system as well.

In conclusion, we demonstrated the effect of 5-HT depletion on the RMP, rheobase and total spikes on nociceptive TG neurons by using patch-clamp recording technique. Even so, 5-HT depletion seems not to be a sole element in migraine pathophysiology. Further patch-clamp recording study on TG neuron under a more specific migraine model should be addressed for the more understanding of electrophysiological changes of this first-order neuron in migraineurs.

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Table 1 RMP and AP properties of TG neuron from the four groups. All values were shown as mean±SD, *p<0.05 by using one-way ANOVA.

Parameters	PCPA-SM (n=9)	PCPA-L (n=11)	Control-SM (n=10)	Control-L (n=8)	p-value
RMP (mV)	-51.15±8.88	-64.71±7.04	-55.82±7.82	-59.24±9.61	0.007*
Capacitance (pF)	59.74±29.32	102.65±66.70	60.83±31.36	70.11±26.32	0.105
Threshold (mV)	-40.63±7.70	-34.96±7.48	-42.35±9.03	-30.92±18.69	0.134
Rheobase (mV)	13.88±11.93	43.18±24.72	16.00±10.75	34.38±17.20	0.001*
AP height (mV)	101.64±10.95	101.48±14.49	97.53±9.42	99.55±17.20	0.887
AP overshoot (mV)	63.63±6.37	62.74±17.75	61.46±14.92	63.98±12.44	0.980
AP rising time (msec)	1.17±0.64	0.85±0.47	0.90±0.58	0.67±0.23	0.246
AP falling time (msec)	1.09±0.41	0.95±0.58	1.24±0.83	0.75±0.32	0.354
AP duration (msec)	2.27±0.78	1.80±0.73	2.14±1.02	1.42±0.52	0.135
AHP depth (mV)	-13.29±7.52	-16.50±10.65	-13.26±10.40	-17.87±7.87	0.649
AHP duration (msec)	0.24±0.65	0.65±1.12	0.17±0.19	0.08±0.01	0.271
Total spike	227.11±210.41	14.64±5.43	137.20±151.79	11.88±4.6	0.002*

Fig. 1 Morphology of neuron from the PCPA-SM (a), PCPA-L (b), control-SM (c) and control-L group (d), scale bars are 5 μm long.

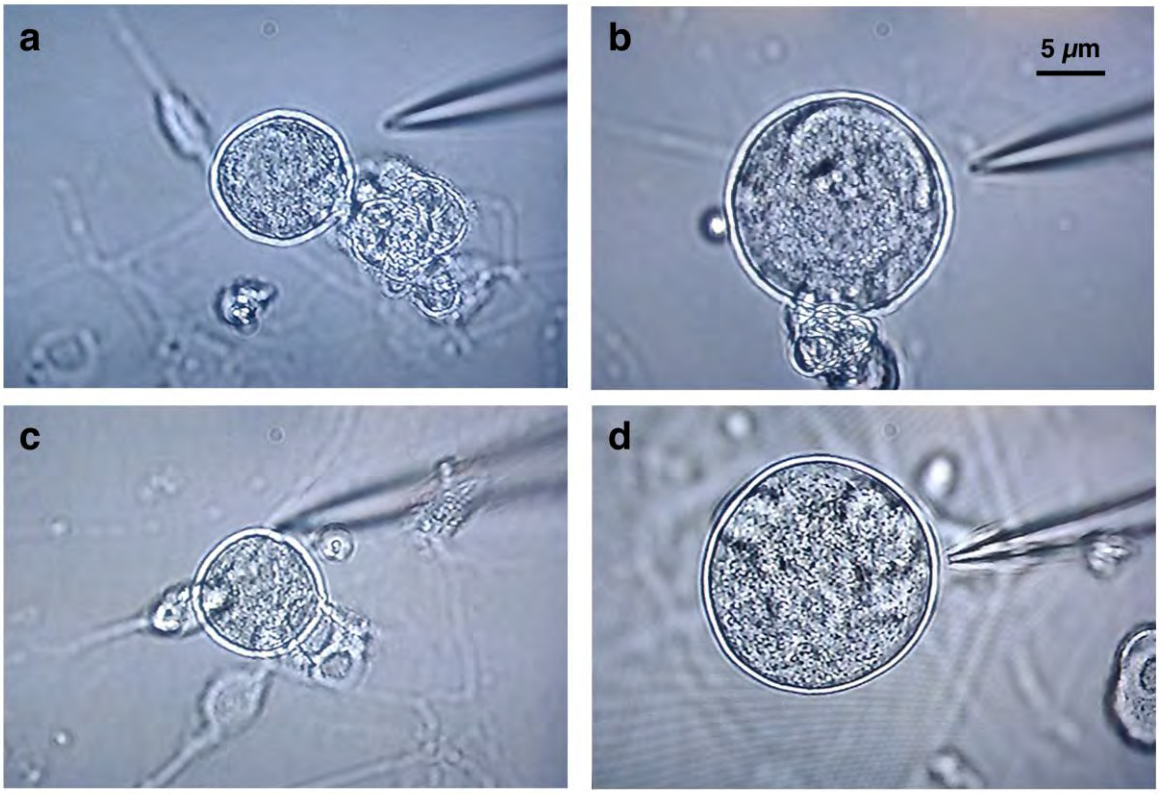


Fig. 2 Representative traces of AP train in TG neurons during evoked-current stimulations. (a) PCPA-SM, (b) PCPA-L, (c) control-SM, (d) control-L.

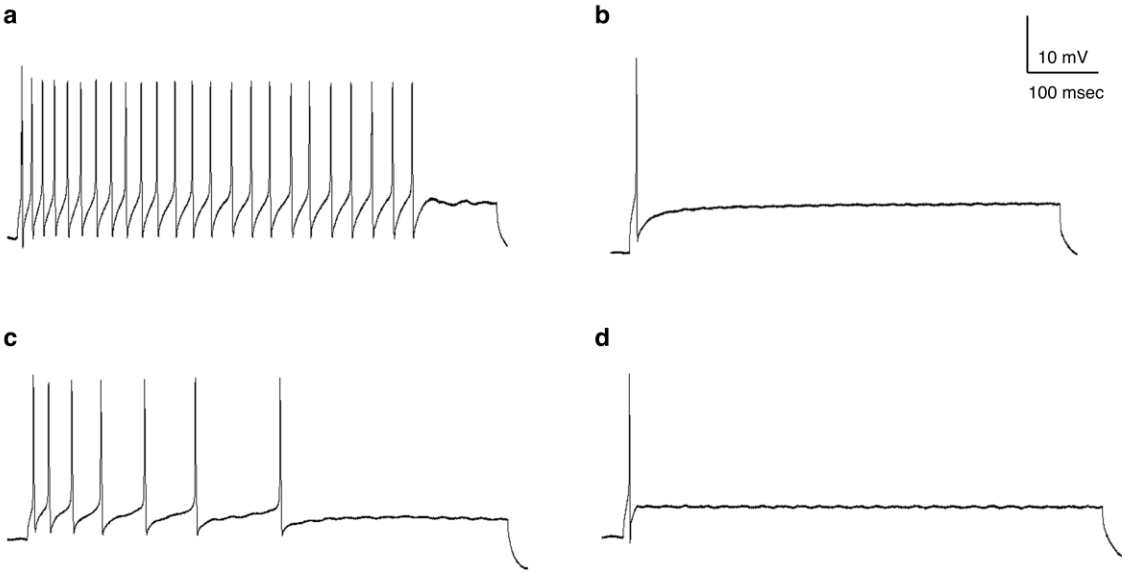
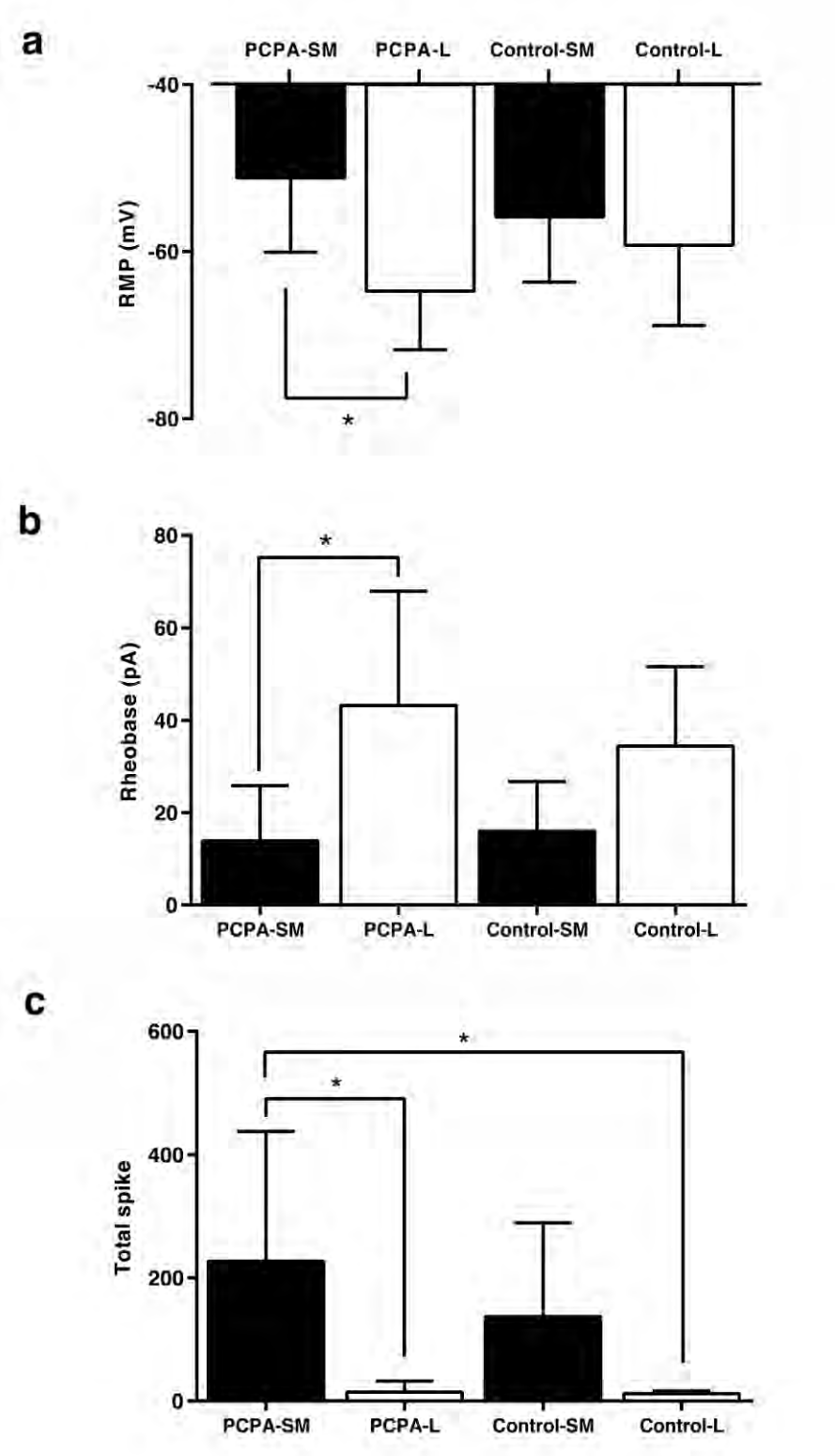


Fig. 3 Electrophysiological parameters of TG neurons showing significant difference among PCPA-SM, PCPA-L, control-SM, control-L groups by ANOVA. (a) RMP, (b) rheobase, (c) total spikes), *p<0.05 when using post-hoc test to determine intergroup differences.



EGFR cut-off point for prognostic impact factor in laryngeal squamous cell carcinoma

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ABSTRACT

IMPORTANCE: In this modern era, various molecular biomarkers for instance epidermal growth factor receptor (EGFR) has gained much importance recently as a predictor of head and neck cancer disease progression.

OBJECTIVES: To investigate the ability of epidermal growth factor receptor (EGFR) expression as a prognostic marker for laryngeal squamous cell carcinoma (SCC).

DESIGN: Retrospective cohort study since January 1, 2010 through December 31, 2014. The demographic data and immunohistochemistry were analyzed.

SETTING: Department of Otolaryngology, King Chulalongkorn Memorial Hospital (KCMH), Bangkok, Thailand.

PARTICIPANTS: Patients with laryngeal SCC treated according to their stages of disease at KCMH in the duration mentioned above were included in this study with total of thirty-one patients.

INTERVENTIONS: Immunohistochemistry staining for EGFR was performed by two pathologists blinded to the patients' information. The digital image processing was applied to analyze EGFR staining intensity and percent distribution which was calculated as H-score.

MAIN OUTCOME MEASURES: We used the receiver operating characteristic (ROC) curve to identify the best cut-off point of EGFR expression using H-score into high and low grade for recurrent prediction of the cancer. Chi-square test was used to evaluate the correlation between the recurrence of disease and EGFR grading. Survival analysis was presented by Kaplan-Meier method.

RESULTS: A total of 31 patients with primary laryngeal SCC were included in this study. There were 30 males and 1 female. Average ages was 66.2 years. The cut-off point for EGFR as high and low grades was H-score of 170 with sensitivity of 75% and specificity of 66.7%. Using this cut-off, 14 (45.16%) and 17 (54.84%) patients were categorized as high and low EGFR grades, respectively. The analysis showed significantly reverse correlation between expression grade of EGFR and recurrence of laryngeal SCC (RR, 0.4; 95%CI, 0.17-0.98; P=.020). Also, high grade EGFR showed tendency of longer 5-year survival compared to the low grade.

CONCLUSIONS: Our study demonstrated that EGFR grading using H-score with the generated cut-off point by ROC curve might be further applied as a novel and potential marker for laryngeal SCC prognostic prediction.

KEYWORDS: EGFR, Laryngeal squamous cell carcinoma, Immunohistochemistry, H-score, Receiver operating characteristic curve, Prognostic factor

INTRODUCTION

In the new era of precision medicine, targeted therapy has become an evolutional role as an adjunctive treatment of HNSCC. In order to make appropriate clinical management decision, standardized prognostic tool is essential. Nowadays, worldwide accepted prognostic parameter of head and neck squamous cell carcinoma (HNSCC) including laryngeal cancer has not yet been generated. Many studies reported the association between endothelial growth factor receptor (EGFR) expression and HNSCC. However, valuing EGFR as a standard prognostic parameter and standard measurement for HNSCC is still inconclusive.

The prognostic value in terms of disease recurrence and survival with EGFR grading in laryngeal squamous cell carcinoma are still controversial. A correlation between high

EGFR expression and poor prognosis including overall survival has been reported.[1,2,3] In contrast, others showed that detection of EGFR amplification resulted in longer overall survival.[5,6] Besides the prognosis, tumor stage is another factor that has been debated in terms of relation with EGFR.[4]. EGFR expression in high-grade invasive cells is significantly downregulated compared with that of low-grade invasive cells.[4] However, some studies showed correlation of EGFR overexpression and stages of the disease.[9,10]. Therefore, there is still inconclusive outcome of survival and stages in different grades of EGFR.

Many previous studies has graded EGFR expression in order to predict the severity and response of disease to the given therapy.[1,3,7,8]. Most of the studies classified the EGFR level into high and low grades and used these grading as a prognostic parameter of HNSCC. [3,8,12] H-score is being use in various reports as one of the methods for grading the EGFR expression.[1,3,7,8] Nevertheless, standard cut-off point for EGFR expression using H-score had still not been established. Some previous squamous cell carcinoma articles have mentioned choosing the EGFR cut-off point arbitrarily.[1] While many others used the cut-off point of H-score 200 according to their outcomes of interest in order to distinguish between high and low EGFR.[3,7,8] However, no specific detail of their methods was shown.[3,7,8] Currently, there was a report of using EGFR expression as an indicator to predict the response of non-small cell lung cancer to the given targeted therapy.[11] They used H-score and the ROC curve as a tool for determining an optimal cut-point for further clinical decision based on the EGFR immunohistochemistry result.[11]

This present study is conducted in order to develop the clinical interpretation of EGFR grading in HNSCC using H-score and generating cut-off point of H-score by applying ROC curve to create a readily available tool as a practical prognostic parameter in both the survival rate and recurrent rate of HNSCC.

MATERIAL AND METHODS

Inclusion criteria for patient selection in this study were as follows: (1) presentation at King Chulalongkorn Memorial Hospital (Tertiary care center) from January 1, 2010 to December 31, 2014 with a diagnosis of squamous cell carcinoma of larynx. (2) treatment according to their stages of disease including radiotherapy, chemoradiation and surgical treatment. A review of medical records found 87 patients who met the inclusion criteria. Of these, 30 had incomplete follow-up, 12 tissue samples were inadequate and 14 referred with pathological report, leaving 31 patients who were retrospectively analyzed without missing data. Tumor samples were obtained from surgical specimens before the chemotherapy and radiotherapy were applied. Hematoxylin and eosin-stained sections were reviewed. Clinicopathological parameters were summarized in **Table 1**.

Immunohistochemistry

Sections from formalin-fixed paraffin embedded blocks of laryngeal cancer tissue were cut at 3 μm thickness. Antigen retrieval was performed by heating sections in an autoclave at 120°C for 10 minutes, followed by incubation with 2% normal bovine serum to block nonspecific staining. Sections were then incubated with an antihuman monoclonal antibody against EGFR 3C6 (Ventana, Tuscon, AZ, USA) at 4°C overnight, followed by incubation with a biotinylated secondary antibody (Antimouse IgG, 1:200; Vector® Laboratories, Burlingame, CA, USA) and Vectastain Elite ABC reagent (Vector® Laboratories) at room temperature for 30 minutes each. Lastly, the sections were treated with 3,3′-diaminobenzidine tetrahydrochloride, followed by counterstaining with hematoxylin. Positive control was stained in normal skin.

Scoring

The stained slides were evaluated by 2 pathologists which blinded the clinical information. All EGFR-stained slides were digitally imaged by the Aperio ScanScope XT

(Aperio Technologies, Vista, CA). The membrane algorithm version 9.1 from Aperio ImageScope v. 12.1.0.5029 was used for quantitative scoring. The score was classified in quantitative number 0-100.

Statistical analysis

The EGFR H-score was calculated using the formula 1 x (percentage of weakly stained cells, 1+) + 2 x (percentage of moderately stained cells staining, 2+) + 3 x (percentage of strongly stained cells, 3+). The optimum cut off point in a ROC curve was analyzed to classified EGFR expression into high and low EGFR expression.

Data analysis was calculated by SPSS version 23. In the evaluation of the data, frequency distributions, means, standard deviations and cross-tables were used. In categorical comparisons, Pearson's chi-squared and Fisher's exact tests were used to analyze the correlation between categorical variables. Kaplan-Meier curve was performed for survival analysis. The log-rank test was performed to compare survival curves between two groups. The significance of all the tests was evaluated at the level of 95% ($p < 0.05$).

RESULTS

Descriptive statistic

A total of 31 patients with primary laryngeal cancer were included in this study. All were eligible for analysis. There were 30 (96.77%) males and 1 (3.23%) female. Average ages in this study was 66.2 years with maximal and minimal ages of 80 and 43 years, respectively. The correlation between EGFR grading and clinicopathological characteristics including histological differentiation, T stage, LN metastasis, stages of disease, tumor recurrence and 5-year survival were demonstrated in **Table 1**. There were 2 patients that were excluded in the topic of 5-year survival shown in Table 1 due to loss follow-up. EGFR grading showed no significant correlation with genders and ages. EGFR grading showed significant correlation with recurrence of disease and 5-year survival. However, EGFR

grading was not related to histological differentiation, T stage, lymph node metastasis, stages of disease, and 5-year survival.

The mean of EGFR expression level among stages of HNSCC patients are significantly difference (p = 0.013) (Data was not shown). The mean EGFR expression was 184.39 ± 29.51, 165.10 ± 114.26, 212.35 ± 30.48 and 137.22 ± 43.38 for stage 1, 2, 3 and 4, respectively. The mean of EGFR expression in stage 4 was lowest and significantly difference compared to the mean of stage 1 (-47, 95%CI -84 to -10; p = 0.013) and to the mean of stage 3 (-75, 95% CI -125 to -25; p = 0.005)

Determining the cut-off level by roc curve

ROC curve of EGFR expression was illustrated in **Fig. 2.** The ROC curve was plotted from the sensitivity and 1-specificity of every possible cut-off points. In ROC analysis, the optimum cut-off point of EGFR expression in order to distinguish between the recurrent and non-recurrent laryngeal cancer was 170 which demonstrated the sensitivity and specificity of 75% and 66.7%, respectively. Using this cut-off, 14 patients were categorized as high and 17 patients were categorized as low EGFR grade.

Association between EGFR grade and number of recurrence

Our study observed that high EGFR expression grade has lower number of recurrent patients than low EGFR expression. (RR, 0.4; 95%CI, 0.17 -0.98; P=.020). Hence, there was significantly reverse correlation between expression grade of EGFR and recurrence of laryngeal SCC.

A total of 17 patients (55%) with low grade EGFR, 5(16%) were diagnosed as SCC recurrence free, 12 (39%) were recurrent. The other 14 patients (45%) with high grade EGFR, 4 (13%) were recurrent, 10 (32%) were non recurrent.

The 5-year survival by Kaplan-Meier analysis for 31 patient with laryngeal cancer

During the follow-up time of 8 years, recurrence in 16 patients (51.61%) was observed. The total of 10 cases (62.5%) recurred at local site, 4 cases (16%) revealed distant metastasis at skin, lung, chest wall and esophagus while 2 cases (12.5%) recurred at both local and distant organs. In the end of this study, 15 patients (48.39%) died in which 8 cases (53.33%) died from the laryngeal cancer, 1 case (6.67%) died from other causes which was myocardial infarction, and 6 cases (40%) died from unknown etiology. Overall survival in patients categorized as low and high EGFR grade was 41.18% and 64.28%, respectively. The 5-year survival curve was shown in **Fig. 4.** It seemed that EGFR high grade had tendency to survive more compared to the EGFR low grade as 5-year survival in patients with EGFR high grade had survived 71.43% compared to 41.18% in the EGFR low grade. However, the difference between these 2 groups showed no statistical significance ($p = 0.139$).

DISCUSSION

This study demonstrates that EGFR is a favorable marker to predict the prognosis of the laryngeal cancer, independent of the tumor staging. The results suggest that low grade EGFR expression are correlated with poor prognosis including tumor recurrence, overall survival and 5-year survival. Furthermore, the accomplished aim of this study is developing the novel method to create new scoring system for EGFR grading in laryngeal squamous cell carcinoma. Although this study had a low number of sample size, the study still proves to show statistically significant result of association between grading of EGFR expression with HNSCC recurrent rate. Further studies have to be performed in large scale for confirming the results of this study and potentially apply it to predict an association of EGFR grading and treatment responsiveness as well as improve a targeted therapy for laryngeal cancer management. Despite this limitation, this study not only generates the scoring system which

lacks bias of inter-pathologist variation but also is the beginning of applying truly practical HNSCC treatment based on EGFR grading to maximize patient's outcome.

Numerous studies topic of association between EGFR expression and head and neck cancer including the larynx are reported. Nevertheless, the consensus of this association has not yet be established.(15) There was a study conducted in human involving oral squamous cell carcinoma (OSCC) cases showed the association of EGFR overexpression with tumor invasion (13). However, the other study regulated in vitro of OSCC cells line found that EGFR expression in high-grade invasive cells was significantly downregulated compared with that of low-grade invasive cells.(8) The in vitro study finding was consistent with this present study which demonstrated a tendency of downregulation of EGFR level in advanced stage of HNSCC patients. The data in this study provided that the mean of EGFR expression level among stages of HNSCC patients are significantly difference and specifically, in stage 4 was lowest and significantly difference compared to the mean of stage 1 and stage 3. Nevertheless, the distinct organs of carcinogenesis could be the explanation of the incongruous results between oral and laryngeal squamous cell carcinoma in human correlation of EGFR expression with stages. Therefore, further in vitro studies should be done to prove this study finding.

In terms of recurrence, this study specifically discovered that HNSCC patients had significant inverse association between EGFR grade and number of recurrence. This result is consistent with the study of patients with oral and oropharyngeal squamous cell carcinoma expressing high levels of EGFR were predicted as having better locoregional recurrence- free survival.(16) This correlation could be explained with the association of tumor recurrence with treatment resistance and tumor invasiveness. Downregulation of EGFR causing hypoxic tumor microenvironment leading HNSCC patients to resistance against treatment with both anti EGFR-antibodies and radiotherapy results in increasing tumor recurrence.(24) In

addition, in vitro study, low levels of EGFR expression in cell lines of OSCC was associated with the resistance to cetuximab treatment and tumor invasiveness.(25) Other studies conducted on human squamous cell carcinomas also demonstrated that in vitro activation of EGFR improved radiosensitivity.(26,27,28) Those previous studies supported our results. Among diverse methods of EGFR expression assessments, none has propelled to the forefront as standard EGFR evaluation. Our study use EGFR staining intensity along with percentage of stained tumor cells of each intensity in order to calculate into H-score. This scoring system has been used as a grading tool for EGFR expression mainly in non-small cell lung cancer.[20,21,22] Application of H-score in order to classify EGFR expression into different grading is currently being discussed. Some previous articles chose the EGFR cut-off point arbitrarily.[20] While other researches supported the use of H-score cut-off point of 200 because the threshold of 200 showed the best concordance and a better separation of subgroups of low and high EGFR.[21, 22] In our study, cut-off point of H-score was generated using the receiver operating characteristic (ROC) curve. In prior study about investigating the potential of plasma EGFR as a diagnostic marker for head and neck cancer, applied ROC for finding an optimal cut-off point between high and low EGFR level with sensitivity of 76.09% and specificity of 67.27%.[23] Compared with prior studies, our study also revealed acceptable sensitivity and specificity which implies reliable outcomes. In the forthcoming era, applying EGFR level as a prognostic tool of laryngeal cancer needs standard, acceptable, and trustworthy process to categorized the EGFR grade and that H-score based on ROC may be one of the method suggested.

Surprisingly, in this study, both the overall survival and 5-year survival observed is longer in the high EGFR grade patients. Even though it is not statistically significance, the tendency is still strongly shown. There are multiple articles that also proposed the same result as this researches.[13, 14, 16] Prior study indicated that the detection of EGFR gene amplification

showed better 5-year survival rate in patients with squamous cell carcinoma of the nasal cavities or paranasal sinuses.[13] Also it showed no statistical difference between both groups.[13] In addition, from another study demonstrated strongly correlation between the reduced membrane EGFR immunoreactivity of the neoplastic cells revealed shorter overall and disease free survival in oral mucosa squamous cell carcinoma.[14]

In contrast to some other studies showed that EGFR negative patients have significant longer 5-year metastasis-free survival, relapse-free survival and overall survival. The meta-analysis also exhibited the EGFR protein overexpression in laryngeal cancer was correlated with decrease in overall survival.[15] The discrepancies with the previous researches might be attributed from various probable factors. These could be explained by the distinct methods use for EGFR expression evaluation, different therapeutic modalities and broad anatomical variety of squamous cell carcinoma organs involvement. In addition, the appearance of viral infection may influence the expression of the EGFR in squamous cell carcinoma.[17, 18] There is an evidence showed that the presence of HPV in oral and orophayngeal cancer cells decrease the expression of EGFR along with better prognosis.[19] As a result, it could be implied that the conflict with the previous research may be from the influence of HPV infection.

In conclusion, this study proves the correlation of EGFR and prognostic outcome in laryngeal carcinoma patients and also creates a novel cut-off point of H-score in EGFR grading by using ROC curve, in order to easily integrate EGFR grading into clinical practice and as one of valuable applicable prognostic predictors in the current world of precision medicine.

Table and Figure Legend

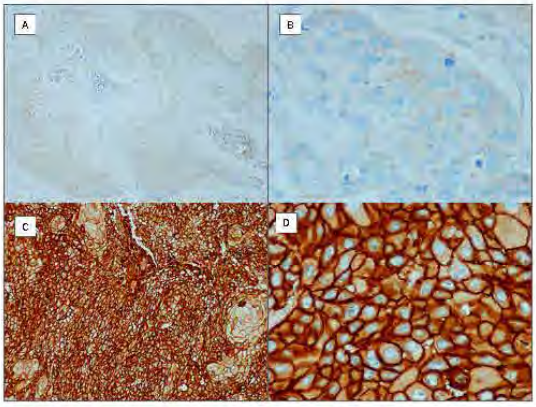
Table 1 Correlation between EGFR expression and clinicopathological features of 31 laryngeal squamous cell cancer patients.

Figure 1 Immunohistochemical staining of epidermal growth factor receptor (EGFR) in head and neck squamous cell carcinoma.(A),(B) Low EGFR expression (X10,40 respectively). (C),(D) High EGFR expression (X10,40 respectively).

Figure 2 ROC curve of EGFR expression. The intersected point between the 2 dash lines representing the best sensitivity and 1-specificity is at the cut-off point of EGFR expression 170. In this model, sensitivity 75% was and specificity was 66.7%.

Figure 3 Significant reverse association between EGFR grade and number of recurrency (*,P=.020)

Figure 4 The 5-year survival by Kaplan-Meier analysis for 31 patient with laryngeal cancer. (p=0.139)


A
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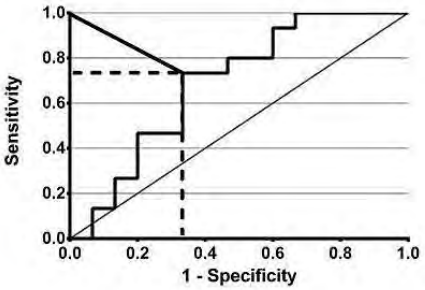
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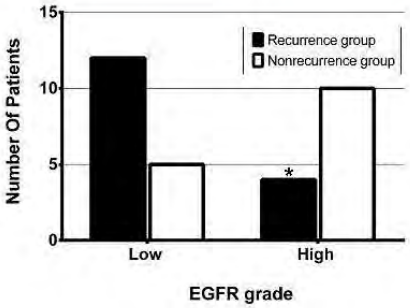
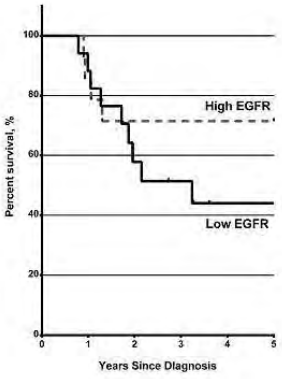

Number Of Patients
Recurrence group
Nonrecurrence group
15
10
5
0
*
Low
High
EGFR grade


Percent survival, %
High EGFR
Low EGFR
100
80
60
40
20
0
0
1
2
3
4
5
Years Since Diagnosis

Physiology Teaching Time Proportion Affects Student's Attitude and Their Self-evaluation Rating

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BACKGROUND: Medical physiology is a branch in biology dealing with various mechanisms in living human, which may look complicated for students in their early years of medical education.[1-2] This study aimed to explore whether time proportion of physiology taught in an organ system block influences student's attitude toward studying physiology and self-evaluation rating about relevant physiology knowledge.

SUMMARY OF WORK: This study was done in medical students during their second-year course from June 2016 to February 2017, in the Faculty of Medicine, Chulalongkorn University, Thailand. All 298 students in the class were enrolled. The students were asked to respond to attitude and self-evaluating questions at the end of 13 physiology laboratory classes throughout the second-year course. Physiology time proportion was defined as the proportion of physiology teaching time to total teaching time in the organ system block responsible for the laboratory class. Relationships of physiology time proportion in the blocks to student's attitude and self-evaluation rating were then explored.

SUMMARY OF RESULTS: Physiology time proportion positively correlated with student's attitude ($r=0.84$, $p=0.001$) and negatively correlated with self-evaluation rating ($r=-0.73$, $p=0.007$).

DISCUSSION AND CONCLUSIONS: Higher time proportion of physiology taught in the block may decrease student's self-evaluation rating regarding relevant physiology knowledge. This can be inferred that it may help reduce the student's overestimation in their academic performance. The higher time proportion may also help promote the student's attitude toward studying physiology.

TAKE-HOME MESSAGES: Higher time proportion of physiology taught in a block may help promote student's attitude toward physiology and may reduce self-evaluation rating in their relevant physiology knowledge.

Keywords: physiology; teaching time proportion; attitude; self-evaluation

References:

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Influences of Attitude and Self-evaluation Rating to Academic Performance in Physiology Learning: A Quantitative Study in Thai Medical Students

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BACKGROUND: Positive attitude was found to be crucial for academic performance.[1-2] While self-evaluation skill is essential attribute for a lifelong learning of medical doctors.[3] Thus, this study aimed to explore the relationship of student's attitude and self-evaluation rating to their academic performance in the context of physiology education for second-year medical students.

SUMMARY OF WORK : A total of 298 medical students were recruited to the study. We organized thirteen physiology laboratory class (LC) sessions. At the end of each LC, the students were asked if they had prepared for the LCs and if they satisfactorily understood content of the LC. These questions are considered as attitude and self-evaluating questions, respectively. Academic performance was determined by formative and summative tests. Based on total score of attitude questions from all LCs, the students were categorized as most pro-active attitude (Most PA), more pro-active attitude (More PA), less pro-active attitude (Less PA) and least pro-active attitude (Least PA) attitude groups. Based on total score from all self-evaluating questions, they were categorized as high (HighE), moderate (ModerateE) and low (LowE) self-evaluation rating groups. Formative and summative scores were then compared among the attitude and self-evaluation categories.

SUMMARY OF RESULTS: The mean formative score of Most PA group was significantly higher than those of all other attitude categories (p=0.003, 0.001 and <0.001), while the mean summative score of Most PA group was significantly higher than those of only Less PA and Least PA groups (p=0.017, 0.015). There was no significant difference of mean formative and summative scores among HighE, ModerateE and LowE groups.

DISCUSSION AND CONCLUSIONS: Positive attitude toward studying physiology may associate with higher academic performance. Differently, self-evaluation rating did not correctly predict the real academic performance, which may be explained by the tendency of self-overestimation in some low-performance students.

TAKE-HOME MESSAGES: Positive attitude toward studying physiology may associate with higher academic performance, while self-evaluation rating did not.

Keywords: physiology; attitude; self-evaluation; academic performance

References:

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Title: Cortical Spreading Depression in Rats Alters Electrophysiological Properties of TG neuron More Than Serotonin Depletion

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Current Affiliation: Department of Physiology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

Abstract: (Within 250 words)

Headache in migraine is generated in trigeminal nociceptive system. Cortical spreading depression (CSD) is a phenomenon responsible for activation of migraine headache, which can be induced in rats by placing potassium chloride (KCl) on their cortical surface. Neurotransmitter changes, especially serotonin (5-HT) depletion in trigeminal nociceptive system, might also predispose migraine. Our study aimed to explore the effects of KCl-induced CSD and 5-HT depletion on electrophysiological properties of trigeminal ganglia neuron. We used male Wistar rats which were divided into four groups. The first was a control group. The second was a 5-HT depletion group, obtained by i.p. injection of p-chlorophenylalanine (PCPA) (100 mg/kg BW). The third was a KCl-induced CSD group. The last group was treated with PCPA to induce 5-HT depletion, following by KCl-induced CSD. After achieving all procedures, both side of TGs were dissected and isolated for recording electrophysiological properties by patch-clamp technique. The results provided important insights in resting membrane potential (RMP) and duration of action potential (AP). RMP (mean±SD, mV) of the 4 groups were -60.39±11.45, -59.77±9.48, -42.70±12.34, -44.51±9.22, respectively (p=0.001). Duration of AP of the 4 groups (mean±SD, msec) were 1.98±1.03, 1.98±0.8, 3.05±2.43, 4.04±2.00, respectively (p=0.002). Our results suggested that KCl-induced CSD had more influence on electrophysiological properties changes of TG neuron than 5-HT depletion.

Biography: (Up to 100 words)

Naeemah Haji-a-sa has been completed her Bachelor of Physical Therapy, School of Health Science, Mae Fah Luang University, Chiang Rai, Thailand. She is pursuing a Master degree of Medical Science at Department of Physiology, Graduate School of Medicine, Chulalongkorn University. Neurophysiology and neuroscience are the fields of interested.

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Session of Interest: Neurons and nervous system
Category: (Oral presentation/ Poster presentation)**:** Oral presentation

Title: The Alteration of Plasma Monoamine Neurotransmitters Level Related to Psychiatric, Sleep, and Sexual Problems in Parkinson's Disease Patients

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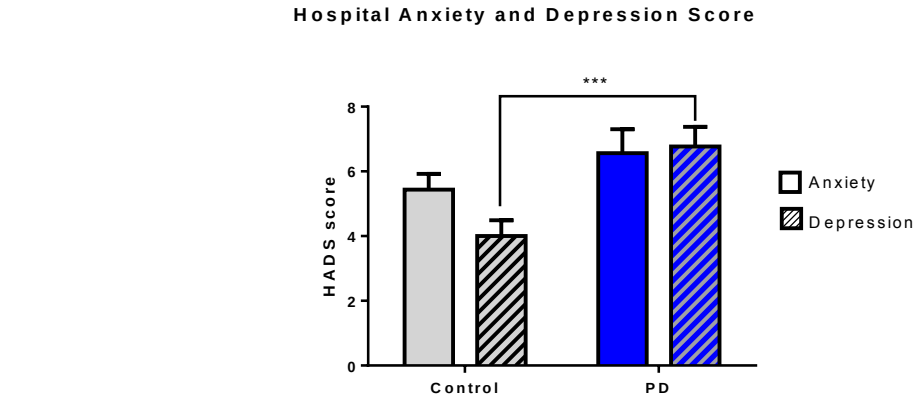
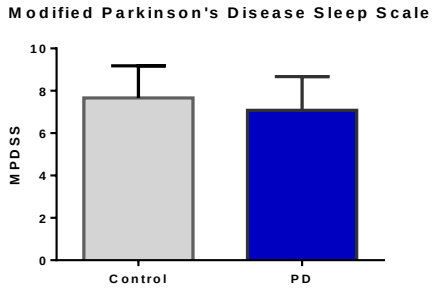
Abstract: (Within 250 words)

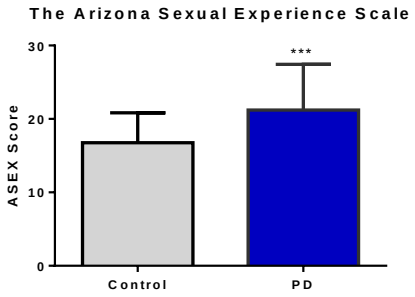
Most Parkinson's disease (PD) patients suffered from a variety of non-motor symptoms (NMSs), which may result from multi-neurotransmitters dysfunction. The purposes of this study were to investigate the alteration of plasma monoamine neurotransmitters (dopamine, norepinephrine, epinephrine, and serotonin) level and their correlation with NMSs in PD patients. Idiopathic PD patients and control subjects (n=30/group) were assessed for NMSs including anxiety, depression, sleep and sexual problems by standard questionnaires, which were the Hospital Anxiety and Depression Scale (HADS), Modified PD Sleep Scale (MPDSS), and the Arizona Sexual Experiences Scale (ASEX), respectively. The plasma monoamine levels were determined by using HPLC with ECD. Then their correlations with NMSs were analyzed. The scores of HADS in depression item and ASEX were significantly higher in PD patients *(p*<0.001). Plasma dopamine (432.40 ± 80.15 versus 169.95 ± 21.03 ng/L) and norepinephrine (1574.92 ± 362 versus 257.37 ± 29.09 ng/L) levels were significantly higher in PD patients than in control subjects. In contrast, levels of epinephrine (527.46 ± 68.01 versus 688.29 ± 53.67 ng/L) and serotonin (11.34 ± 2.56 versus 27.55 ± 6.11 µg/L) were markedly lower in PD patients (*p*<0.001). Importantly, there was negative correlation between plasma dopamine and depression score (r=-0.323, *p*<0.05). From these findings, depression and sexual dysfunction were the obvious problems of PD patients. The alterations of monoamine neurotransmitters were prominent in PD and correlate to some of NMSs, which may be applied as potential biomarkers for NMSs prediction in the future.

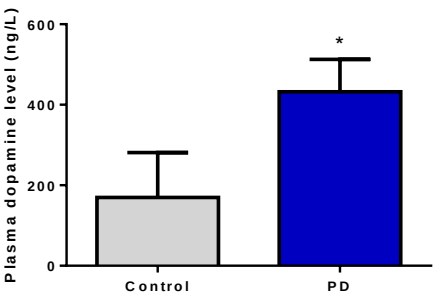
Session of Interest: Neurodegenerative Disorders and Neuro Imaging

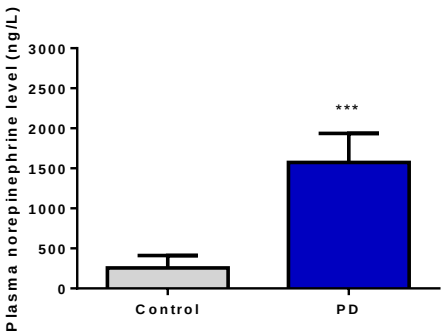
Category: Oral presentation

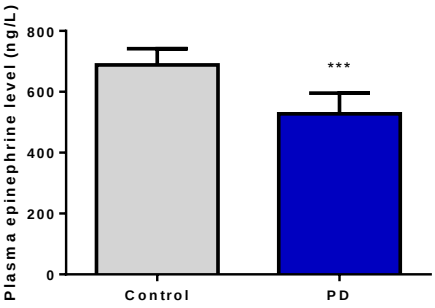

Hospital Anxiety and Depression Score
***
HADS score
8
6
4
2
0
Control
PD
Anxiety
Depression

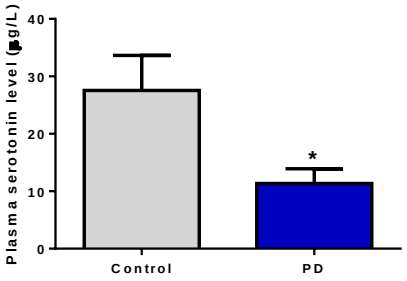

Modified Parkinson's Disease Sleep Scale
MPDSS
10
8
6
4
2
0
Control
PD

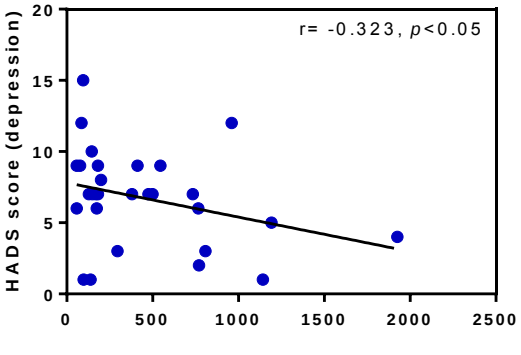

The Arizona Sexual Experience Scale
ASEX Score
30
20
10
0
***
Control
PD


Plasma dopamine level (ng/L)
600
400
200
0
*
Control
PD


Plasma norepinephrine level (ng/L)
3000
2500
2000
1500
1000
500
0
***
Control
PD


Plasma epinephrine level (ng/L)
800
600
400
200
0
***
Control
PD


Plasma serotonin level (ng/L)
40
30
20
10
0
*
Control
PD


r= -0.323, p<0.05
HADS score (depression)
20
15
10
5
0
0
500
1000
1500
2000
2500
Plasma dopamine level (ng/L)

Clinicopathological and prognostic value of EGFR expression in laryngeal squamous cell carcinoma

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Introduction

In an era of personalized medicine, we use various biomarkers which might predict the response of the cancer treatment. Epidermal growth factor(EGFR) is one of them which can develop the targeted therapy. However, the role of EGFR in laryngeal cancer remains controversial. The prognostic role and standard measurement are not fully consensus.

Aim of the study

To determine the role of EGFR and prognostic value in laryngeal cancer with clinicopathological correlation.

Material and method

31 Laryngeal SCCA → Demographic Data, Pathological Data, Clinical F/U 8 years

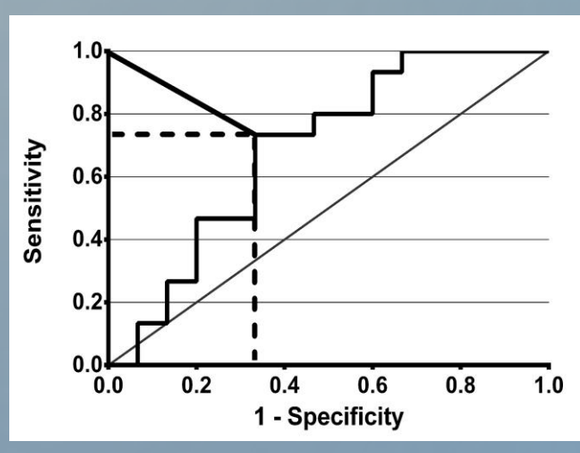
→ EGFR Immunohistochemistry → Digital image analysis → Statistical analysis, ROC curve

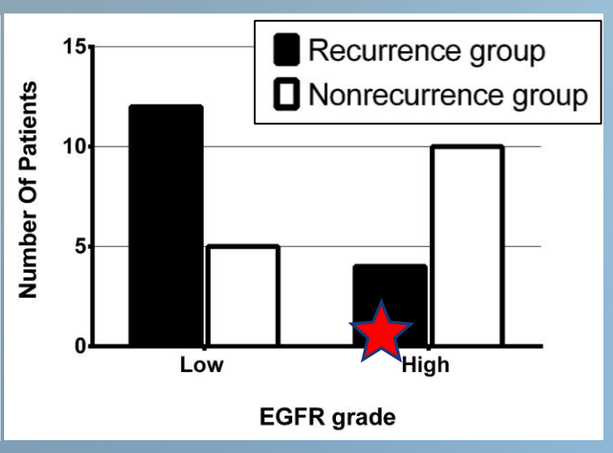
H score = $\frac{1(\text{EGFR1+})+2(\text{EGFR2+})+3(\text{EGFFR3+})+4(\text{EGFR4+})}{10}$

Results

Clinicopathological features		EGFR expression		Total	P-value
		High	Low		
Total		14 (45%)	17 (55%)	31(100%)	
Histological differentiation					
	Well	8 (26%)	10 (32%)	18 (58%)	$X^2 = 0.925$
	Moderate	6 (19%)	7 (23%)	13 (42%)	
Stage					
	I	6 (19%)	3 (10%)	9 (29%)	Fisher $X^2 = 0.202$
	II	1 (3%)	1 (3%)	2 (6%)	
	III	3 (10%)	1 (3%)	4 (13%)	
	IV	4 (13%)	12 (39%)	16 (52%)	
Recurrent					★
	No	10 (32%)	5 (16%)	15 (48%)	$X^2 = 0.020$
	Yes	4 (13%)	12 (39%)	16 (52%)	
Survival					★
	> 5 years	10 (32%)	5 (16%)	15 (48%)	$X^2 = 0.040$
	< 5 years	4 (13%)	10 (32%)	14 (45%)	

★ = differences were statistically significance





- EGFR grading showed significant correlation with recurrence of disease and 5-year survival.
- EGFR grading was not related to histological differentiation, T stage, lymph node metastasis, stages of disease.
- ROC curve of EGFR expression, the best sensitivity and 1-specificity is at the cut-off point of EGFR expression 170. In this model, sensitivity 75% was and specificity was 66.7%.
- Significant reverse association between EGFR grade and number of recurrency (*P*=.020)

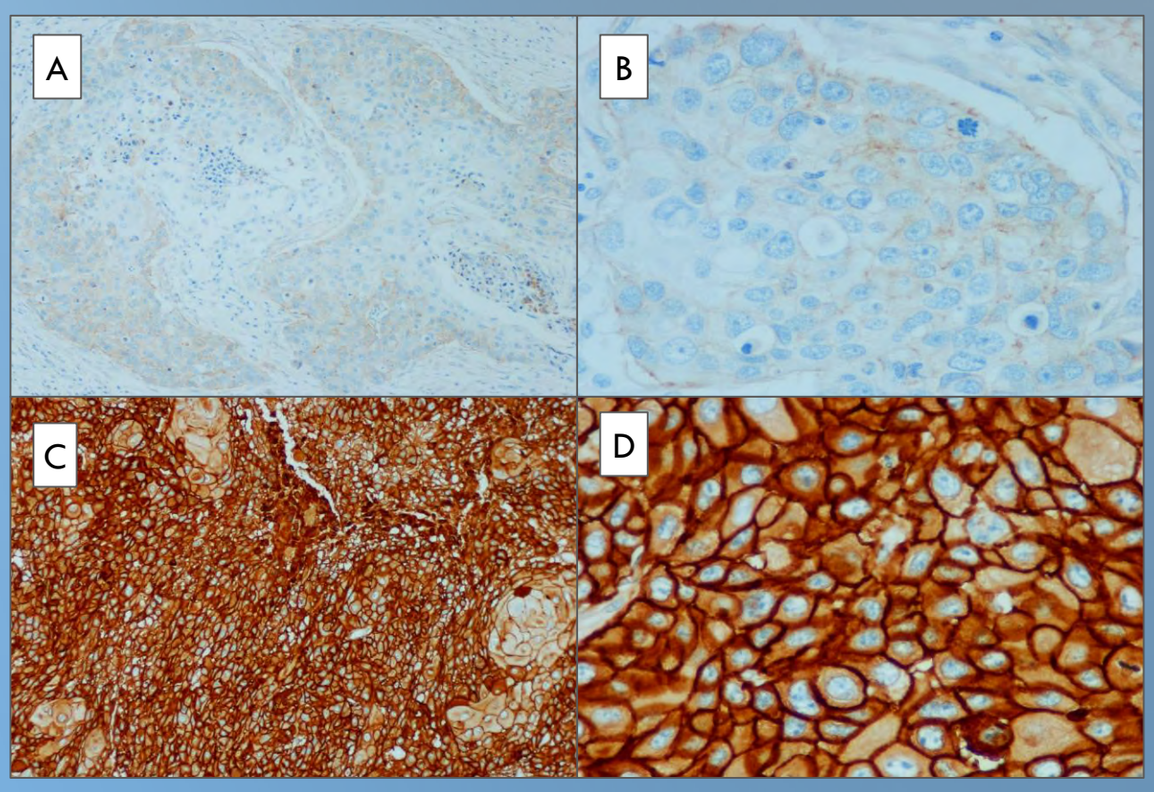


Figure 1. showed immunohistochemistry of EGFR in laryngeal SCCA.
(A),(B) Low EGFR expression (X10,40 respectively)
(C),(D) High EGFR expression (X10,40 respectively).

Conclusion

- EGFR revealed a significant correlation with recurrence of laryngeal squamous cell carcinoma and 5-year survival.
- ROC curve showed the best cut-off point of EGFR expression was 170 from H-score.
- The high EGFR expression had less recurrent rate.
- EGFR expression can be a valued biomarker which can predict the prognosis of laryngeal squamous cell carcinoma.

Acknowledgement

References

1.

Downregulation of EGFR in advanced stage laryngeal squamous cell carcinoma

Vivatvakin S., Ratchataswan T., Leesutipornchai T., Ruangritchankul K., Keelawat S., Kerekhanjanarong V., Mahattanasakul P., Bongsebandhu-Phybhakdi S

***Abstract*— In this globalization era, much attention has been drawn to various molecular biomarkers which may have the potential to predict the progression of cancer. Epidermal growth factor receptor (EGFR) is the classic member of the ErbB family of membrane-associated intrinsic tyrosine kinase receptors. EGFR expression was found in several organs throughout the body as its roles involve in the regulation of cell proliferation, survival, and differentiation in normal physiologic conditions. However, anomalous expression whether over- or underexpression is believed to be the underlying mechanism of pathologic conditions including carcinogenesis. Even though numerous discussions regarding the EGFR as a prognostic tool in head and neck cancer have been established, the consensus has not yet been met.**

The aim of the present study is to assess the correlation between the level of EGFR expression and demographic data as well as clinicopathological features and to evaluate the ability of EGFR as a reliable prognostic marker. Furthermore, we aim to investigate the probable pathophysiology that explains the finding results. This retrospective study included 30 squamous cell laryngeal carcinoma patients treated at King Chulalongkorn Memorial Hospital from January 1, 2000 to December 31, 2004.

EGFR expression level was observed to be significantly downregulated with the progression of the laryngeal cancer stage. (one way ANOVA, $p = 0.001$) A statistically significant lower EGFR expression in the late stage of the disease compared to the early stage was recorded. (unpaired t-test, $p = 0.041$) EGFR overexpression also showed the tendency to increase recurrence of cancer. (unpaired t-test, $p = 0.128$)

A significant downregulation of EGFR expression is documented in advanced stage laryngeal cancer. Our results indicated that EGFR level correlates to prognosis in term of stage progression. Thus, EGFR expression might be used as a novel prevailing biomarker for laryngeal squamous cell carcinoma prognostic prediction.

***Keywords*—downregulation, epidermal growth factor receptor, immunohistochemistry, laryngeal squamous cell carcinoma.**

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Abstract

KCl-induced CSD on electrophysiological properties in primary cultured of trigeminal ganglion neurons

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Objective: KCl-induced Cortical spreading depression (CSD) is an animal model of migraine, it activates and sensitizes to the trigeminal afferent, signal transduction send to trigeminal ganglion (TG). However, the mechanisms of CSD involved to primary cultured of TG neurons is still unknown. Thus, we discover effect of KCl-induced CSD alters pattern of electrophysiological properties in primary cultured TG neurons.

Materials and methods: In this experiment, we divided male Wistar rat into 2 groups. First group, KCl-induced CSD. Second, control group, rat injected with thiopental 70 mg/kg and decapitated to removes both sides of trigeminal ganglia for primary cell cultured. Procedure of KCl-induced CSD done under anesthesia with thiopental 70 mg/kg BW and rat was setting on the stereotaxic frame. Bur hole performed and dura was exposed, and removed. KCl crystal 3 mg subsequently placed into bur hole for 1 hour and sacrificed. We removed both sides of TG for primary cell cultured. Primary cell cultured incubated for 18-24 hours and studied with physiological patch-clamp to discover pattern of action potential. Data presented in mean±SD and performed independent t-test for statistical analysis at p-value ≤ 0.05.

Results: The Capacitance (Cm) of left TG neurons in CSD (23.54 ± 15.97, n=9) was significance lower than control (58.79± 31.52, n=18), p-value= 0.0055. Resting membrane potential (RMP) of left TG neurons in CSD (-37.14 ±12.90) was significance higher than control group (-60.61± 10.06), p-value= 0.000039.

Conclusions: The mechanisms of KCl-induced CSD involved to decrease of Cm contributed to have a transmembrane current increased, result in RMP increased of TG neurons.