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# Induction of Neural Stem Cells on Indium Tin Oxide Surface

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**Abstract**

**Neural stem/progenitor cells (NSPCs) are suitable candidates on the development of neural network engineering. Patterning and differentiated protocols have been tried to develop in order to generate functional neurons and to establish well neural networks.**

**In this study, we fabricated niche-modulated system to investigate effects on NSPCs differentiation by the formation of polyelectrolyte multilayer (PEM) films governed by electrostatic interactions of poly-L-glutamine acid as a polyanion and poly-L-lysine as a polycation. Moreover, since PEM films provide the surface charge effect on the NSPCs differentiation, the indium tin oxide (ITO) surface adjustable electrical stimulation was also applied to model the surface charged property of PEM films. Herein, a serum- and chemical agent-free system provided a clear platform to observe the interaction between substrate niche and stem cell differentiation.**

**Our results revealed that NSPCs were inducible on ITO surface with electrical stimulation and adsorbed with PEM films. The average neurite outgrowth length was over 300 μm after 3 days culture when the electrical stimulation potential was 80 mV. The value was approached that on PEM films. Moreover, the quantity and quality of the differentiated neurons were analyzed by the immunostaining. It is suggested that cell differentiation and process development on ITO surface could be controlled by the electrical stimulation that can be simply adjusted by external equipment.**

## 1. Introduction

Polyelectrolyte multilayer (PEM) films can be constructed based on non-stoichiometric electrostatic interactions and subsequently physical adsorption onto solid surfaces by the alternated adsorption of cationic and anionic polyelectrolyte layers. This widely applicable and promising technique can be used to fabricate films with thickness variation that offer great potential for film property tuning such as composition, hydration, and surface roughness [1,2]. The opportunity of the combination of microelectronics and neural cells has attracted a great attention because it opens a possibility for the research on brain chips and neural engineering. However, the interface between electronic circuit and synaptically connected neurons is a major challenge in the design of the device.

Previous study revealed that the zeta potential of PLL/PLGA PEM film was around the range of 100 mV [3]. Since cell membranes carry negative charges in the serum-free medium, it is considered that cells preferred to adhesive on the positively charged PLL-ending than on the PLGA-ending PEM film. In this study, ITO conductive surface applied with electrical potential was used to provide positive surface charge to model the surface charge effect and to induce NSPCs differentiation. ITO has been widely used as a conductive material in microelectronic circuits, it is a well candidate to be used on neurochip for the study of neural signal transmission in a well-constructed network [4].

In this study, the induction of neural stem/progenitor cells (NSPCs) by an electrically adjustable indium tin oxide (ITO) conductive surface and ITO-PEM films were investigated. In addition, the biological and differentiation effects of two systems on NSPCs, including cell cytotoxicity, neurite outgrowth length, differentiation lineage, and synapse functionality of differentiated neurons were determined in this study.

## 2. Method of approach

*Experimental design of ITO-PEM films and ITO conductive surface applied with electrical potential*

A biochamber was fabricated to investigate the induction effect of NSPCs. A layer of polydimethylsiloxane material with four culture chambers was sandwiched by two flat ITO-glass substrates to form a closed cell culture system and the NSPCs were maintained at 37ºC in a humidified 5% $CO_2$ incubator. Cells were cultured in the chamber on different conditions, including ITO-glass with electrical stimulation and PEM film/ITO-glass. The electrical stimulation was shown as Fig.1 (A). NSPCs stimulations by sequential potentials from 0 to 100 mV across the electrodes were observed. For the design of the electrical stimulation of the biochamber, a uniform parallel electric field was generated by a pair of parallel plates. In addition, the PEM film was built up by the alternate adsorption of PLL and PLGA as shown in Fig.1 (B).

*Preparation of ITO-PEM films*

Physical deposition of PEM films was performed by batch and static conditions as follows: initially, all polypeptides were dissolved in 10 mM Tris-HCl buffer with 0.15 M NaCl, pH 7.4. ITO glass was then immersed in PLL solution for 10 min, followed by rinsing with Tris-HCl buffer. Then, the PLL-adsorbed ITO glass were subsequently immersed in PLGA solution for 10 min, followed by rinsing with 1 mL of Tris-HCl buffer for 1 min. Lastly, substrates were cleaned with fresh PBS to remove uncoupled polypeptides.

*Isolation and culture of cortical NSPCs*

Cerebral cortical NSPCs were isolated from ED 14-15 Wistar rat embryos using a previously defined protocol with modification [5].

*Lactate dehydrogenase activity assay*

The cytotoxicity of electrical stimulation system and ITO-PEM films to NSPCs was determined with a cytotoxicity detection kit (Roche, Mannheim, Germany). The operated procedure was followed the manufacturer's protocol. LDH content was assessed by enzyme-linked immunosorbent assay (ELISA) and read at an absorbance of 490 nm in a plate reader with a reference wavelength of 630 nm.

*Immunocytochemistry, process length, and differentiation percentage of neural cells*

For immunocytochemical characterization, cells cultured for 5 days in vitro were fixed in ice-cold 4% paraformaldehyde in PBS for 20 min and washed three times in PBS. After fixing, cells were incubated with the following primary antibodies for 2 h at 37ºC. The cells were then incubated with secondary antibodies for 30 min at room temperature to visualize the signal. Immunostained cells were visualized by a fluorescence microscope. Quantification of process length after 3 days culture and differentiation percentage after 5 days culture were collected by using Image J software

## 3. Results

The quantification of process length of electrical stimulation is shown in Fig. 2(A). The process length increased with the increase of the applied potential from 0 to 80 mV, but reduced at 100 mV. The optimal electrical stimulation was found to be 80 mV that could stimulate the longest process. This result was reasonably aligned with the previous study [3]. The zeta potential induced by PEM film on glass substrate was around 80 mV on positively charged PLL-ending films when the number of layers was higher than 3. Alternatively, the result of PEM film is shown in Fig. 2(B). The process lengths were increased as the layer increased. In addition, the process lengths in different intervals which revealed that the potential of 80 mV could stimulate the longest processes (601-900 μm) (0-300, 301-600, and 601-900 μm) (not shown here).

## 4. Conclusions

This study proposed to use ITO-glass as the culture substrate with adjustable electrical stimulation to enhance the cell attachment and control the neurite outgrowth. In comparison with ITO-PEM films system, our results revealed that electrical stimulation could also strongly control the NSPCs differentiation behavior and generated the functional neurons.


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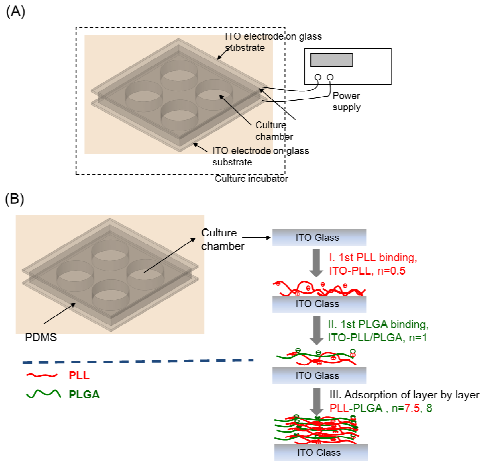


Fig. 1 Design of the comparative experimental study of differentiation of NSPCs. (A) Illustration of the electrical stimulation biochamber. (B) Illustration of the ITO glass-PEM biochamber.

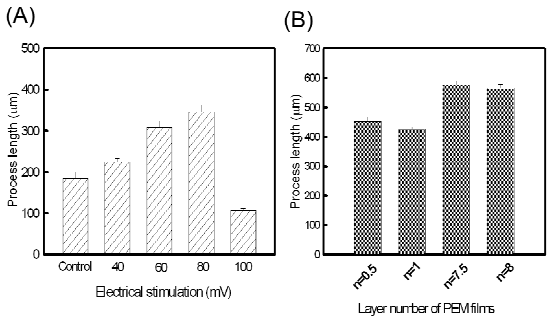


Fig. 2 Quantitative study of process lengths of neurospheres under serum-free conditions at 250 neurospheres per $cm^2$ after 3 days culture. The lengths of the 10-15 longest processes per neurosphere were estimated from the edge of the neurospheres to the tip of the processes in linear form. (A) NSPCs were cultured on bare ITO-glass with different electrical stimulation. (B) NSPCs were cultured on ITO glass-PEM films layers number of n = 0.5, 1, 7.5, and 8.

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