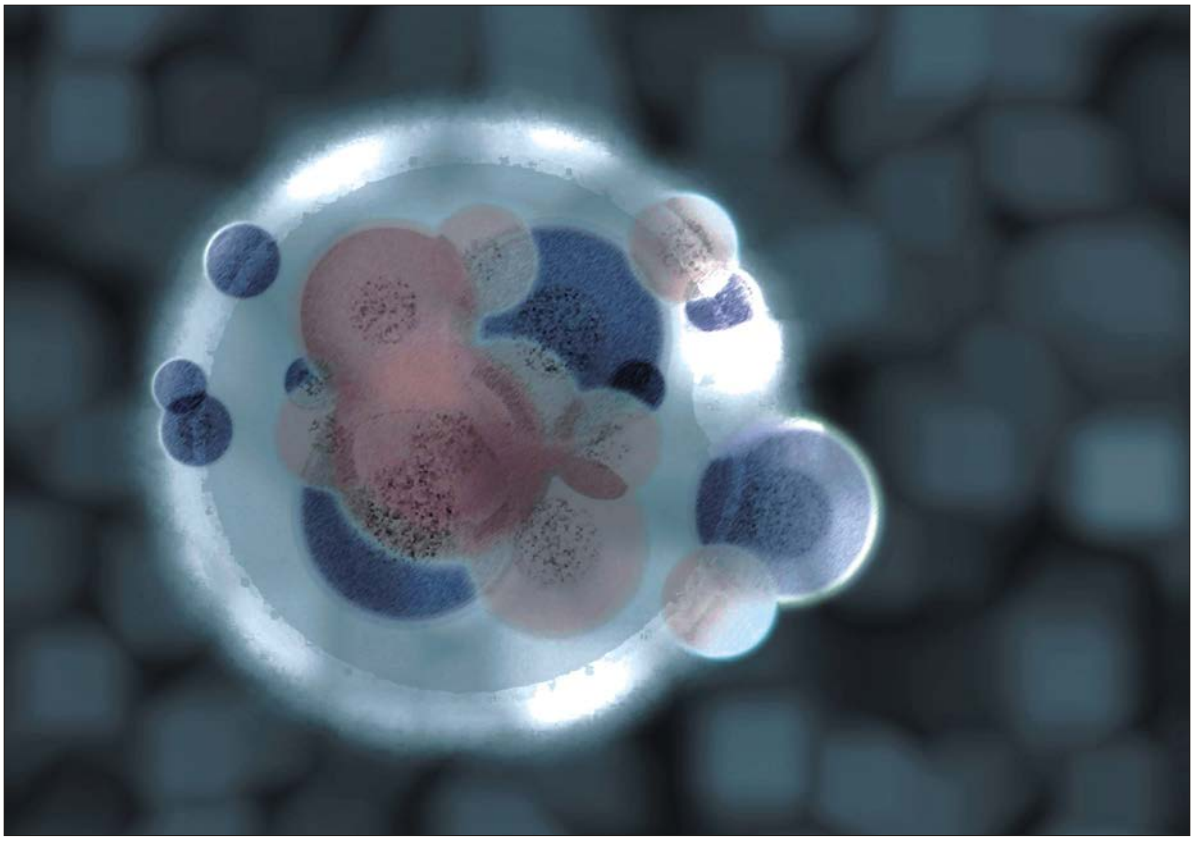

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Research Article
Investigation of Electroporation Technique on Cell Properties Cultured on Self-assembled Monolayer

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Abstract
Electroporation (EP) is a method of controlling cell function by using pulses of electrical fields to create pore through a cell membrane and causes other substance around it to be absorbed into the cell. Where this method had been led to a variety of medical applications. While, micro contact printing (μCP) is a quite useful technique for patterning extracellular matrix as an adhesion molecule for cells that works for controlling the cell growth. This study focuses on a comparison of a cancer cell (HeLa cancer cell) cultured on two different type of substrates which is a protein surface and empty surface. In order to see the effect of cell proliferation of cancer cell with protein solution, which is in this experiment we used fibronectin for the protein solution and the preliminary result show a positive respond to the protein solution that act as self-assembled monolayer.

Key words: μCP, Hela cancer cell, fibronectin, SAMs, PDMS

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Microcontact printing has been developed about 17 years ago and it is an outstanding surface patterning technique in micron scale and also in nanoscale[1]. Surface science communities such as engineers and biologists have been promoting attention in μCP and therefore enriching in improvement to the μCP process itself[2]. The μCP is a soft lithography that used the release pattern on master polydimenthylsiloxane (PDMS) stamp to form a patterns of self-assembled monolayers (SAMs) of ink on the surface of a substrate through conformal contact[3]. In the original version of μCP, the micro-metre-scale patterned chemical modification of a large surface area is obtained by transferring different types of compounds using a soft polymer stamp[4]. Electroporation or electropermeabilization is a method to introduce molecules or a method for increasing cell membrane permeability to molecules. It had been used in various biotechnological and biomedical applications, such as the introduction of molecules into cells, cell fusion, tissue ablation and sterilization of water and liquid food. In molecular medicine and biotechnology, tissue electro-poration is performed with electrodes placed in the target area of the body[5]. Electroporation can be endorsed in 3 type of ways that is *ex vivo*, *in vivo* and *in vitro*. Cell electroporation *in vitro* is used mainly for transfection by DNA introduction but many other interventions are possible, including microbial killing. *Ex vivo* electroporation provides the manipulation of cells that are reintroduced into the body to provide therapy[6]. *In vivo* electroporation of tissues enhances molecular transport through the tissues and into their constitutive cells. By applying an electrical pulses across cells it can have a variety of outcomes from the result; from no effect to a reversible electroporation to irreversible electroporation[7].

MATERIALS AND METHODS

Cell lines and culture conditions: In this study, HeLa cells were used. HeLa cells are cultured in standard culture flask in Rosewell Park Memorial Institute (RPMI) 1640 media (Sigma) with 10% fetal bovine serum until 90% confluence. The cell was maintained in the atmosphere of 5% CO_2 at 37°C and were split once reaching confluence, usually every 5-6 days[8] (Fig. 1).

Microcontact printing method: Figure 2 shows the concept of the fabricating high resolution patterns of protein using a μCP method with a PDMS stamp start[9] at A and B. Where, A show step of inking the PDMS stamp with protein solution

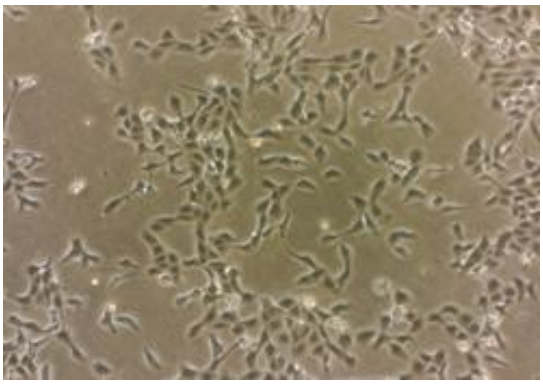

Fig. 1: HeLa cell with 50% confluence

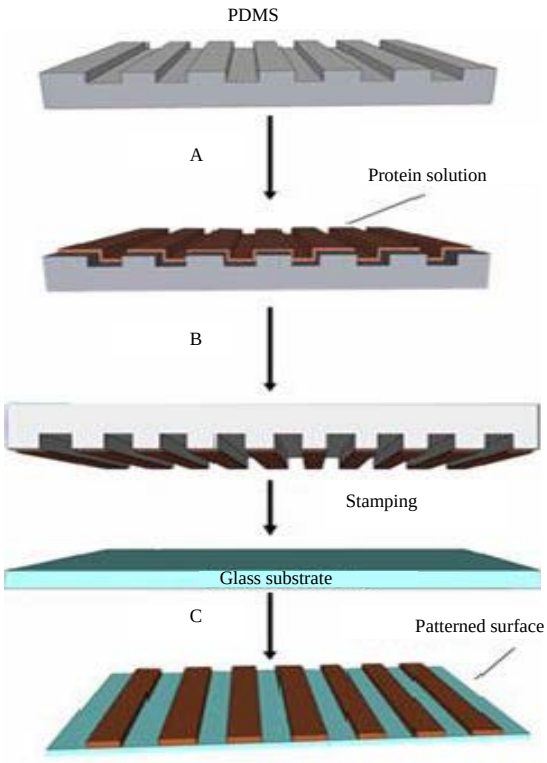

Fig. 2: Process flow schematic of microcontact printing method

and B shows the transferring the proteins to a substrate area or contact or it called step of stamping PDMS with protein at the new substrate by applying a small amount of force and C shows a patterned surface after remove the stamp from the stamping step, the ink is patterned on the glass substrate[10].

Electroporation technique: For the electroporation technique, Fig. 3 and 4 shows the main components of the experimental setup. A nikon inverted research microscope

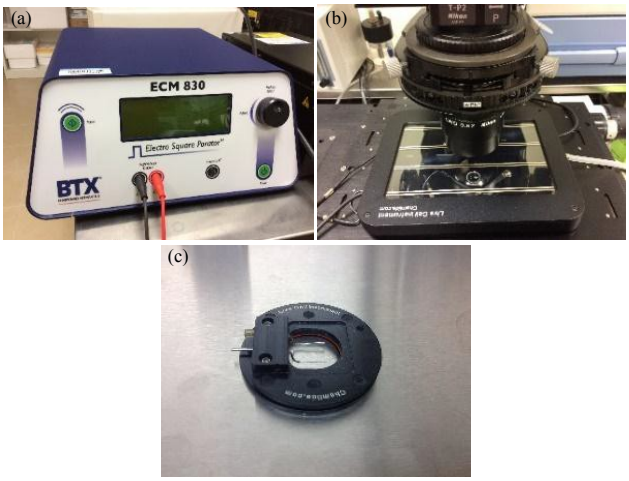

Fig. 3(a-c): (a) High voltage pulsed system (ECM® 830), (b) Chamlide TC stage and (c) Microchamber slide (Chamlide EC)

Fig. 4: Nikon inverted microscope (Ti series)

(Ti series) offers improved speed, increased flexibility and efficient multi-mode microscopy as part of fully integrated microscope system for cell live imaging. In order to expose biological cells to high voltage pulse electric field, two main subsystems are needed: (1) Imaging chamber system (Chamlide EC) which is connected with the main components, dedicated to the applications of field stimulation, which supplied with a pair of platinum electrodes[11] and (2) High voltage pulsed system (ECM® 830) which allow to deliver 10-600 µsec duration of pulses with the adjusted amplitude upto 3 kV[12].

RESULTS AND DISCUSSION

Patterning test with Fetal Bovine Serum (FBS) proteins: Fetal Bovine Serum (FBS) is used initially to test µCP

functionality as in protein patterning. Figure 5 shows that the protein patterning outcome by using microcontact printing method with variety size such as 100, 50, 25 and10 μm. The variety size of stamp used to identify which pattern size will be suitable in microcontact printing method to analyze the cell alignment activity close to 100%.

Electroporation effect on HeLa cell cultured on protein surface and empty surface: With the availability of the HeLa cancer cell, this test was conducted in order to see the effect of the electroporation technique on HeLa cancer cell with the protein solution, which is in this experiment we used fibronectin and the electroporation technique we used

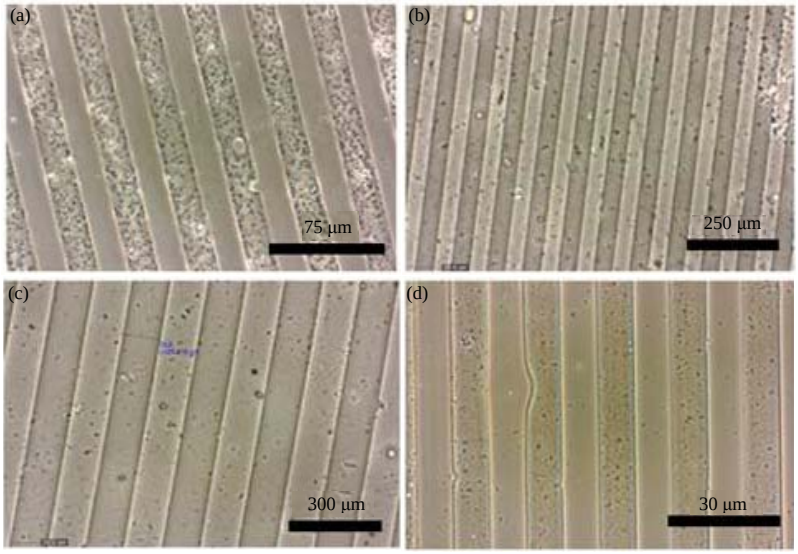

Fig. 5(a-d): Patterns of proteins by microcontact printing method with variety of size, (a) 75 μm, (b) 250 μm, (c) 300 μm and (d) 30 μm

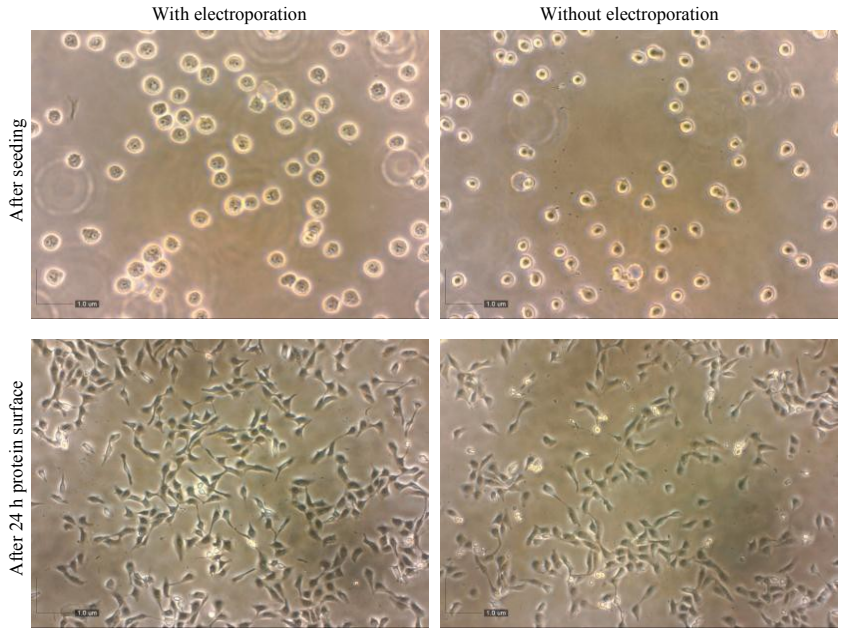

Fig. 6: Continue

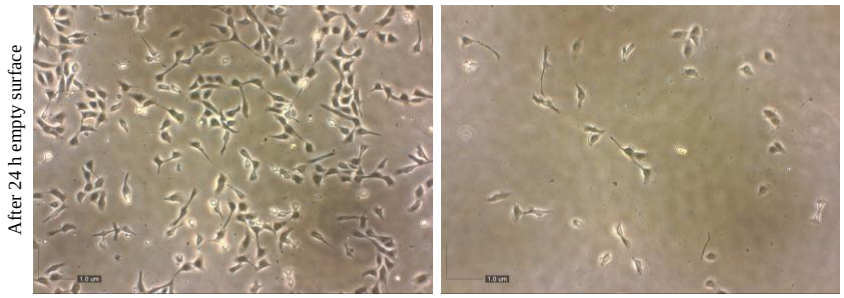

Fig. 6: Electroporation effect on HeLa cell within 24 h

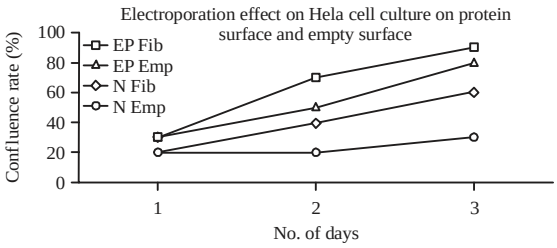

Fig. 7: Graph shows a confluence rate of HeLa cell in this test

2000 kV with pulse length of 30 µsec in order to achieve high electrical field intensity. Where in this experiment, in one well, it divides into two sections of the substrate. Which is the left side is the empty surface while the right side is the protein surface (Fig. 6).

It had been left for 24 h to see the effect of the electroporation technique on HeLa with the protein solution. After 24 h, the result as Fig. 7 shows that the proliferation rate of HeLa cell with EP is increased dramatically at 24 h compared with HeLa cell without EP and also most of HeLa cell attracts with protein surface with the ratio confluence 3:7. This shows that HeLa cell can be used in the fabricating process on the next objective of this study.

CONCLUSION

In conclusion, the preliminary result shown in the previous section, confirm the cell adhesion and proliferation occurs mainly on the fibronectin coated area. By comparing the previous research on the microcontact printing and electroporation, there is minor study on the combination of these two method. The basic concepts and techniques of electroporation and microcontact printing were highlighted. Both EP and µCP were found to be related to wound healing process, depending on the level of their threshold and application (gene therapy, electrochemotherapy and wound healing or tissue ablation). Thus, investigations of EP and µCP showed that the two methods can be combined in *in vitro* to see the cell response to µCP with the PEF applied to it. Important parameters (pulse number and pulse duration) should be considered along with MCP method/technique, which include the cell type and system configuration. We strongly believe that the result of this study can lead to the development of wound healing and cancer treatment.

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