Dielectrophoresis-induced Cell Patterning Using a New PLA Scaffold made by 3D Printing*

Zhijie Huan, Henry K. Chu, Jie Yang and Dong Sun

Abstract— **This paper presents a new technique of fabricating 3D-printed scaffolds that can utilizes dielectrophoresis (DEP) for cell patterning. The scaffold was first fabricated using a 3D printer with a biodegradable polymer, polylactic acid (PLA). The electrical conductivity of the polymeric scaffold was enhanced through sputtering a thin layer of gold. When a voltage was supplied to the scaffold, non-uniform electric fields were generated so that cells were polarized and patterned onto the scaffold. Experiments were conducted to demonstrate that the gold-coated PLA scaffold could be used for rapid patterning MC3T3-E1 cells via DEP. Cell proliferation was assessed by (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) MTT method and the result confirms the DEP cell patterning mechanism is not cytotoxic.**

I. Introduction

The use of polylactic acid (PLA) for biomedical applications has received increasing attention in recent years. Comparing to other typical polymers such as polystyrene and polyethylene, PLA is a naturally biodegradable material as its raw material can be synthesized through the fermentation of starchy materials or sugar [1]. In addition, PLA has excellent mechanical properties and processing capabilities [2]. Combined with rapid prototyping techniques, various PLA microstructures with well-defined geometries can be easily produced with high precision [3-5].

Scaffolds are temporary microstructures that play a very important role in cell and tissue cultures. Depending on the intended application, the geometry of the scaffold should be fabricated accordingly in order to provide a more favorable environment for the cells to be seeded and grow gradually. Nevertheless, conventional cell seeding techniques usually have limited or no controllability on the cell distribution [6]. In this paper, we proposed a new scaffold fabrication technique that can enable active cell seeding via dielectrophoresis (DEP). Scaffolds with the desired geometry were first fabricated with a commercial 3D printer. Computer simulation was performed to confirm the electric field distribution of the proposed scaffold design. To enable cell manipulation and patterning via dielectrophoresis, the PLA scaffold was sputter-coated with a thin layer of gold. Experiments were conducted to evaluate the proposed scaffold design on different aspects. First, the conductivities of the different sputter-coated scaffolds were examined. Then, the proposed scaffold was tested with MC3T3-E1 cells to examine the effectiveness of cell manipulation via dielectrophoresis. Finally, the effect of DEP patterning on the cell proliferation was assessed through MTT assay.

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Z. Huan is with the Department of Mechanical and Biomedical Engineering, City University of Hong Kong, Kowloon, Hong Kong, and the Department of Precision Machinery and Instrumentation, University of Science and Technology of China, Hefei, Anhui, China (email: zhijhuan@cityu.edu.hk).

H. K. Chu is with the Department of Mechanical Engineering, The Hong Kong Polytechnic University, Kowloon, Hong Kong, China (email: henry.chu@polyu.edu.hk).

J. Yang is with the Department of Precision Machinery and Instrumentation, University of Science and Technology of China, Hefei, Anhui, China(email: jieyang@ustc.edu.cn).

D. Sun is with the Department of Mechanical and Biomedical Engineering, City University of Hong Kong, Kowloon, Hong Kong (email: medsun@cityu.edu.hk; Tel: 852-3442-8405).

II. Materials and Methods

A. Principle of dielectrophoresis (DEP)

DEP has been widely used as a non-invasive technique for manipulating batches of cells [7]. This phenomenon was firstly discovered by Pohl [8]. When a polarizable particle is placed in the non-uniform electric field, DEP force will be induced onto the particle. For a spherical particle, the DEP force is determined as follows [9]:

$$F_{DEP} = 2\pi r^3 \varepsilon_m \,\mathrm{Re}[K(\omega)]\nabla\left|\vec{E}\right|^2 \qquad (1)$$

where ε_m is the real part of the permittivity of the suspending medium, r is the radius of the particle, $\nabla\left|\vec{E}\right|^2$ is the gradient of the square of electric field, and $\mathrm{Re}\left[K(\omega)\right]$ is the real part of the Clausius-Mossotti (CM) factor.

Depending on the CM factor, the particle is either trapped or expelled by the DEP force under the positive (p-DEP) or (n-DEP) phenomenon. In this work, the scaffold was designed such that a large number of cells could be trapped and patterned by p-DEP effect.

B. Scaffold design and fabrication

A commercial 3D printer utilizing the fused deposition modeling (FDM) technology was used to fabricate the proposed polymeric scaffold. PLA filament was selected as the 3D ink of the M3D printer. According to the manufacturer's specification, the printer's nozzle has a diameter of 350 microns and the selectable layer resolution is between 50 to 350 microns. In this work, a scaffold with a zig-zag groove was considered and examined for DEP cell

patterning. The dimensions of each triangle along the groove are approximately 2mm by 2mm by 2.83mm, with a thickness of 3mm. The 3D CAD model of the scaffold was built using SolidWorks and the model in STL format was then imported into the machine for printing. Since the 3D printing material is not conductive by nature, the printed scaffold was coated with a conductive material to enhance the electrical conductivity.

According to the principle of DEP, the scaffold should be able to generate high electric field gradients along the zig-zag groove, as shown in Figure 1, so that cells could be patterned onto the scaffold. COMSOL was used to simulate the electric field distribution when an AC function generator was connected to the scaffold and the result confirms that high electric field gradients can be generated at the triangular tips of the groove, as shown in Figure 2.

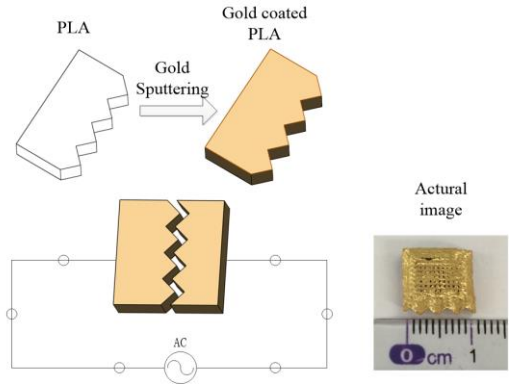


Figure 1. Schematic illustration of the DEP device

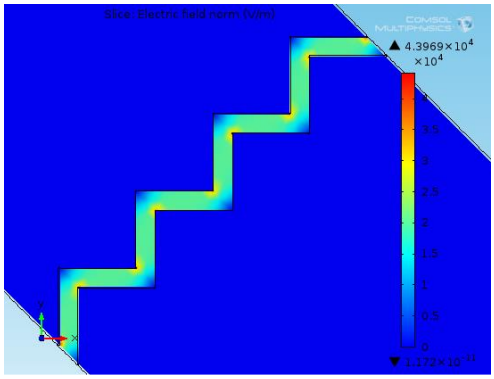


Figure 2. Electric field simulation with COMSOL

C. Cell preparation

Preosteoblast MC3T3-E1 cells derived from mouse calvaria were selected for cell patterning with the proposed scaffold. The cells were cultured in a 35 mm Petri dish in Dulbecco's modified Eagle's medium (DMEM) containing 10% (v/v) heat-inactivated fetal bovine serum (FBS) and 1% (v/v) penicillin–streptomycin. The Petri dish was incubated at 37 °C with a gas mixture of 95% air and 5% CO_2 and the culture medium was changed every two days.

Prior to the experiment, MC3T3-E1 cells were harvested from the culture dish. The cells were washed with phosphate-buffered saline (PBS) and then trypsinized using a 0.25% trypsin–EDTA solution. The cells were then detached from the Petri dish and then transferred to a centrifuge tube to obtain the cell pellet. In order to obtain fluorescent images of the cellular patterns, the cells were stained with MitoTracker Red for 30 minutes. The culture medium was aspirated and a low-conductivity medium (8.5% sucrose, 0.3% dextrose, 20 mg/L $CaCl_2$) was used as the DEP buffer medium [10], so that the cells are more polarizable than the buffer medium, inducing a p-DEP effect.

D. Cell proliferation assay

To evaluate the influence of the DEP force on MC3T3-E1, MTT assay was used to detect the cell metabolism after DEP patterning. Droplets of the cell containing medium were added to the zig-zag groove of the scaffold. After DEP manipulation and patterning for 10 minutes, the patterned cells were collected and transferred to a centrifuge tube for medium exchange. The cell pallet was aspirated with the culture medium and then divided into five parts for subsequent culture in a 96-well plate. The same amount of cells without DEP effect was also cultured in the 96-well plate and served as the control group. The plate was cultured in the incubator for 48 hours. Then, 100µL MTT solution was added to each well and the plate was further incubated for 4 hours at 37ºC. Afterwards, MTT solution was removed and 100µL Formazan Solubilization Solution was added to each well to dissolve the formazan crystals. The absorbance value was measured using a microplate reader (SpectraMax M5e Microplate Reader) at 570 nm.

III. Results and Discussion

A. Conductivity measurement

In order to improve the electrical conductivity of the 3D printed scaffold for DEP patterning, the PLA scaffold can be coated with a conductive layer. Three different biocompatible materials, carbon, titanium and gold, were considered as the target for sputtering, and they were coated onto the scaffold using a sputter coater, Quorum Q150TS. The thickness of the conductive layer was set to be 50nm for all three materials. A digital multimeter (SANWA) was used to measure conductivity of the material after coating. The resistance values were measured at 5 different locations on the surface of the scaffold and Table I summarizes the average resistance values from the scaffolds with and without coating. As presented in [11], a highly conductive scaffold is desired as it could reduce the voltage required for DEP cell manipulation. Based on the result, gold was chosen as the ideal target for sputter-coating the PLA scaffold as it has the lowest resistance. The coated scaffolds were also studied with an immersion test. After immersing the scaffolds in the culture medium for 2 hours, the scaffolds were air-dried thoroughly and the

resistance values were measured again. From the results, the resistance of the gold-coated scaffold is comparable to value from the non-immersed scaffold, indicating the stability and suitability of coating material for DEP cell patterning.

Table I. RESISTIVITY OF THE MATERIAL

	PLA	PLA (carbon)	PLA (titanium)	PLA (gold)
Resistance(Ω)	∞	∞	10k	26.5
Resistance(Ω), after 2 hours of immersion	∞	∞	11.6k	28

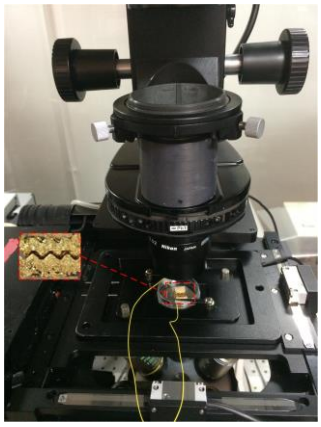


Figure 3. Experiment setup for DEP manipulation

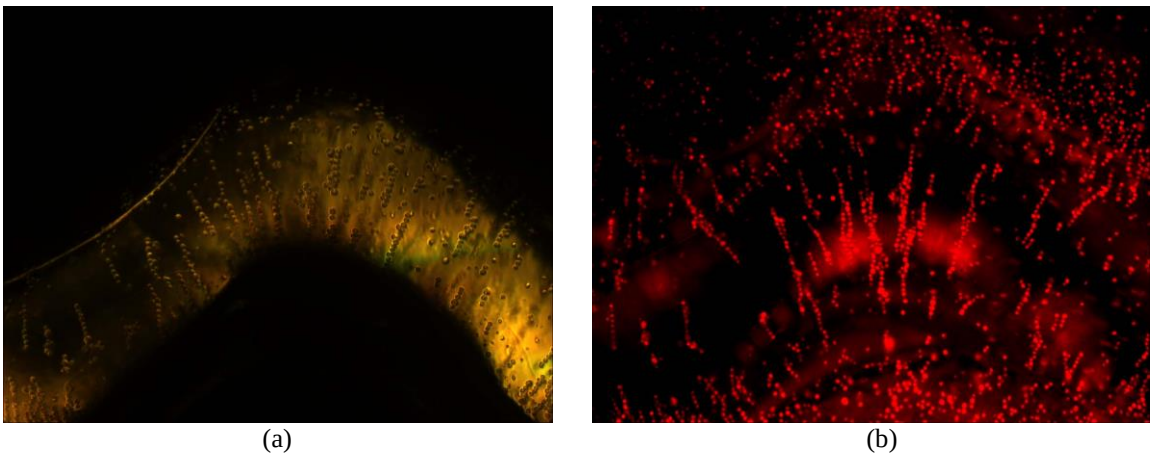
B. Cell patterning via dielectrophoresis

To examine cell manipulation via dielectrophoresis, an AC function generator, GW Instek, GFG8255A, providing a voltage range of 0 to 10 V and a frequency range of 0 to 5MHz, was connected to the scaffold through the two wires as shown Figure 3. During the experiment, a sinusoidal voltage of 5 V at 500k Hz was supplied to the scaffold to generate the required electric fields along the zig-zag groove. Droplets of cell containing medium, at a concentration of approximately 4×10^6 cells/mL, were added to the scaffold and the formation of the cellular pattern was observed by an inverted microscope system, Nikon Eclipse Ti.

Once the voltage was applied to the scaffold, the suspended cells were immediately patterned under the DEP force. Cell chains could be observed along the groove after 5 minutes of operation, as shown in Figure 4.

C. Cell proliferation after DEP patterning

In order to examine the biological properties of MC3T3-E1 after the DEP patterning, MTT assay was further used to measure the cell metabolism. The patterned cells were collected and cultured for 2 days. The proliferation of MC3T3-E1 cells with and without DEP effect was then measured. Figure 5 illustrates the final absorbance as mean value ± standard deviation.



(a) (b)

Figure 4. Image of the patterned cells under the inverted microscope: (a) bright field image; (b) fluorescent image

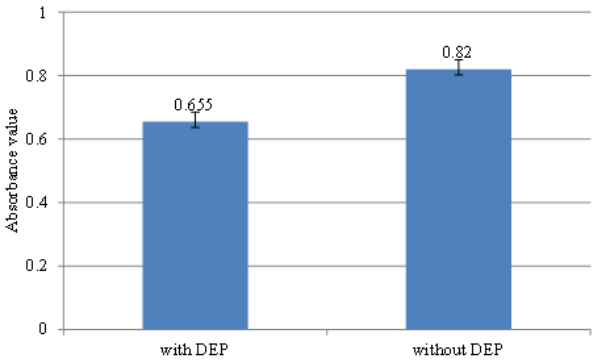


Figure 5. Cell proliferation of MC3T3-E1 cells with and without DEP effect after 2 days of culture.

According to ISO 10993-5 [12], the reduction of cell viability with DEP compared to the control group after 2 days could be calculated as:

$$Viab.\% = \frac{100 \times OD_{570DEP}}{OD_{570C}} \quad (2)$$

where

OD_{570DEP} is the mean value of the measured optical density of the samples with DEP effect, which is 0.655.

OD_{570C} is the mean value of the measured optical density of the control group, which is 0.82.

The calculated viability appeared to be 79.9%, indicating that the cell manipulation mechanism via dielectrophoresis does not induce cytotoxicity to the cells.

IV. Conclusion

This paper presents a new technique for fabricating 3D-printed scaffolds that can utilizes dielectrophoresis for cell patterning. Scaffolds with a zig-zag groove were examined in this work and the scaffold was fabricated from a commercial 3D printer using a biodegradable material, PLA. The scaffold was then coated with a gold layer to improve the electrical conductivity. Experiments were conducted to show the coated scaffold possesses excellent electrical conductivity, enabling cells to be patterned in a short period of time using dielectrophoresis. The cytotoxicity of the DEP cell patterning mechanism was also examined and confirmed to be not cytotoxic. The proposed fabrication technique thus offers a new way to design and develop complicated 3D scaffolds with DEP cell patterning for high-quality tissue reconstruction.

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