MICROFABRICATED PARTICLE ASSEMBLIES FOR VERSATILE CELL PATTERNING

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ABSTRACT

This paper reports a unique method to fabricate microstructures made up of polymeric particles which allows spatial patterning of live cells on planar as well as curved surfaces. The technological combination of electrospray and microfabrication enables the construction of microporous particulate structures on the cell culturing substrates; while the use of floating electrodes greatly enhances the patterning contrast and leads to superior 3D patterning capacity.

KEYWORDS: Microparticle, Electrospray, Floating electrode, Cell patterning

INTRODUCTION

Spatially controllable positioning of living cells has attracted great attention due to the broad applications in fundamental cell biological studies [1], cell-based sensing [2] as well as tissue engineering [3]. Researchers have developed a number of cell manipulation methods such as optical tweezers, acoustic tweezers, dielectrophoresis (DEP), and hydrodynamic flows. Apart from these physical methods, the development of microfabrication technologies in the past decade has largely enriched cell patterning methods by introducing precise surface engineering, in which the spatial patterning of living cells is confined by regulating surface chemistry. Among these methods relying on chemical modification, microcontact printing (μCP) along with its derivative techniques are most widely used, especially for biomolecule and cell patterning. Although these methods have provided plenty of essential insights into cellular functions, cells are often patterned on a planar surface, which is different from the situation in physiological environment where cells often reside on 3D substratum (e.g. endothelial cells on the inner wall of blood vessel, and epithelial cells on the intestine wall).

To circumvent this limitation, we employ standard electrospraying method towards spatial patterning of polymeric microparticles. The electric connection states of individual microelectrodes on the collecting surface are controlled to spatially confine the microparticles. In particular, the floating electrodes are used to enhance the patterning contrast, which allow patterning of microparticles on the planar surface or along the vertical direction. The applications of this unique method including spatial control of cell adhesion as well as patterning live cells into 3D features are demonstrated. With the superior 3D patterning capacity and the simple configuration, this work is expected to facilitate the development of biosensing, microscale tissue engineering and other microparticle-based total analyses.

EXPERIMENTAL

The polymer solution was prepared by mixing 10% (w/w) PCL with acetone. Experimental set-up of the electrospray process is shown in Figure 1. A DC voltage bias of 20kV was applied while the PCL solution was pumped through a metallic capillary at a flow rate of 1.5ml/hr. Linear and circular interdigitated microelectrodes array fabricated by standard photolithography were used as the collecting substrates. Under the electrostatic field, the polymer droplet at the capillary tip was deformed into a conical shape. When the electrorepulsive force overcame the surface tension, a liquid jet was ejected, forming a stream of small and highly charged liquid droplets. The solvent in the droplets evaporated while the droplets were flowing towards the collecting surface positioned at 15cm vertical distance from the capillary tip. Polymeric microparticles were then formed as the solvent evaporated, and were eventually deposited on the collecting surface (Figure 2).

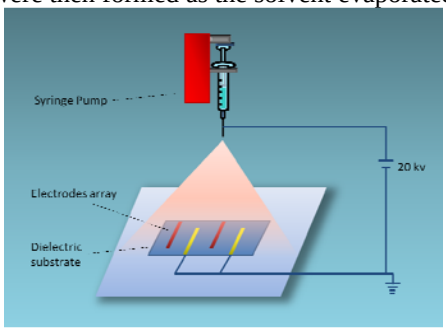

Figure 1: Experimental setup for electrospray process.

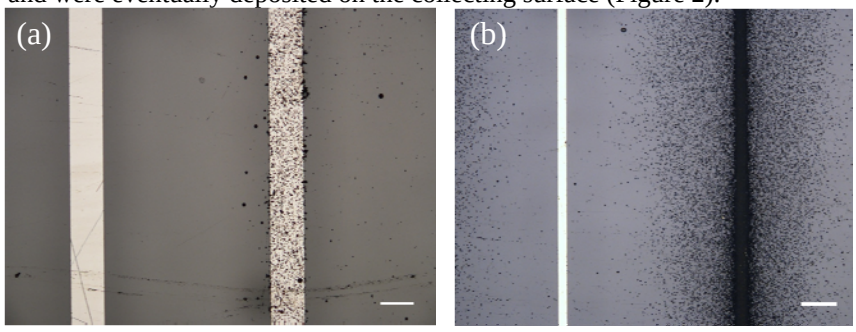

Figure 2: Spatial distribution of microparticles after (b)30s and (c)120s electrospray. The right electrode was grounded while the left electrode was kept floating. Scale bar=200μm.

NIH-3T3 fibroblasts cells were incubated at 37°C under 5% CO_2 atmosphere. The culturing medium was Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum and 1% penicillin/streptomycin. Before cell seeding, the glass substrate patterned with microparticles array was incubated at room temperature in a solution of 3% bovine serum albumin in PBS for 30 min. Subsequently, the cells were trypsinized from a culture flask and diluted with medium to the concentration of 1×10^6 cells/ml. The cells were then seeded on top of the pretreated porous scaffold.

After 72 hr culture, cells were fixed and stained with rhodamine-phalloidin for labeling actin filaments (10min, 25°C), and 4',6-diamidino-2-phenylindole (DAPI) for labeling cell nucleus (15min, 25°C). Visualization of cell patterning was carried out using widefield fluorescence microscope (Eclipse 80i, Nikon, Japan) and confocal microscope (FlouView FV1000, Olympus, Japan). Rhodamine-phalloidin was excited at 540nm and the emission wavelength was chosen at around 625nm. For DAPI, the excitation and emission wavelength was 350nm and 470nm, respectively. The bright field images and the associated fluorescent images were overlapped using the NIS-Elements AR software.

RESULTS AND DISCUSSION

The principle of particle patterning and the role of the floating electrodes were discussed in our previous work [4]. Briefly, the collecting substrate with microelectrodes can pattern microparticles due to the locally non-uniform electric field they arose; and the floating electrodes can effectively enhance the patterning contrast. In this study, electrospray was performed by dividing the microelectrodes into two electrical connection groups. The odd numbered electrodes were grounded while the even numbered electrodes were floated from the ground level. Finite element analysis on the electric potential in the region close to the collecting surface showed that a potential well with steep slope was formed above each ground electrode (Figure 3a), suggesting that in presence of floating electrodes positively charged microparticles may experience an electrostatic force pushing them towards the ground electrodes (or away from floating electrodes). The modeling results indicated that particles which entered the near-electrode region from the top boundary were repelled by the floating electrodes and ultimately deposited on the ground electrodes (Figure 3b).

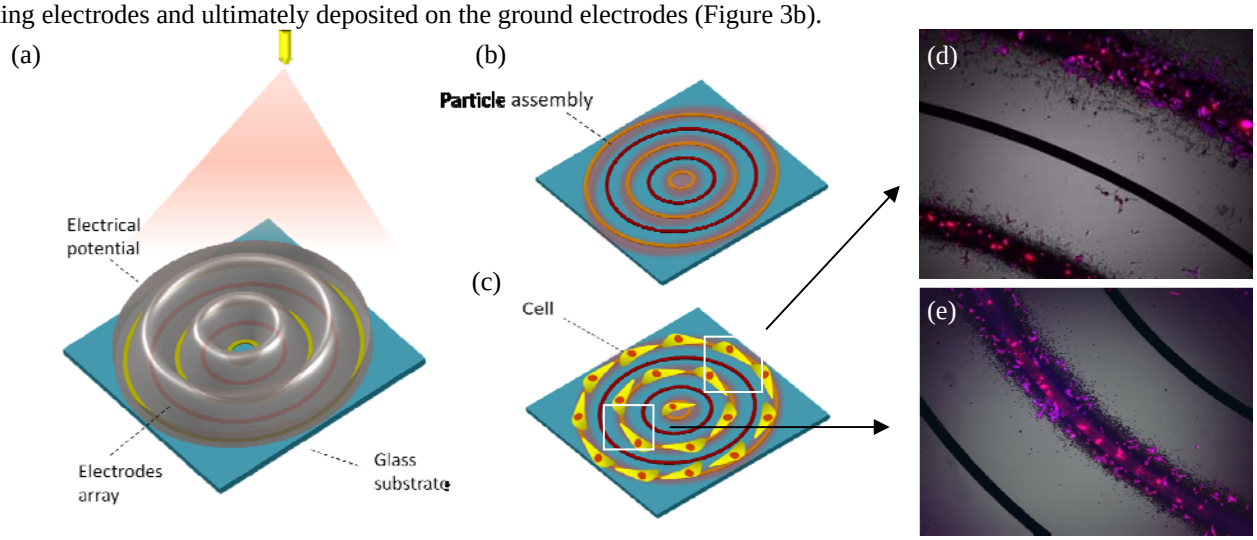

Figure 3: 2D particle patterning with the assistance of floating electrodes. (a)-(c)Schematics of cell patterning process. The yellow circle denote the ground electrodes. The red blocks denote the floating electrodes.(d)&(e) Fluorescent micrographs showing the cell patterning on a planar surface.

The patterning capacity was experimentally examined. Most microparticles were deposited on the ground electrodes after a 30 sec electrospray, while very few particles were observed on the floating electrodes and the inter-electrode area (Figure 2a). The 120 sec electrospray resulted in a similar particle distribution (Figure 2b): the particle density in the inter-electrode area was significantly lower than that in the electrode area, and the particle density on the floating electrode was even much lower. Therefore, it was demonstrated that with the assistance of floating electrodes, microparticles can be confined within desired regions with a high patterning contrast.

The patterned monolayer of microparticles was subsequently used as a substrate to direct cell adhesion. Fluorescent micrographs in Figure 3d&e show the fibroblast cells cultured on a BSA modified particle-patterned surface. The cells were denoted by red (actin cytoskeleton) and blue (nucleus) stains. The black solid lines indicate electrodes array and the black dots are microparticles. It is shown that after 72 hr culturing the cells were mainly confined to the particle assembly regions, while little cell attachment was observed on the region between the particle assemblies.

It is known that the curve surfaces of the particles increase the surface area. Yap et al. calculated the amount of such increase over the area each particle occupied on the planar surface [5]. They concluded that the particles brought in about 1.81 times larger surface area, regardless of the size of the particles. This is essentially important for cell adhesion because the in-

creased cell-substrate contact area upgrades the level of integrin recruitment and thus enhances the total amount of focal adhesion per cell. Consequently, the adhesion strength arises and the cell residing on particles assembly is better anchored on its underlying substrate, as demonstrated in the current work.

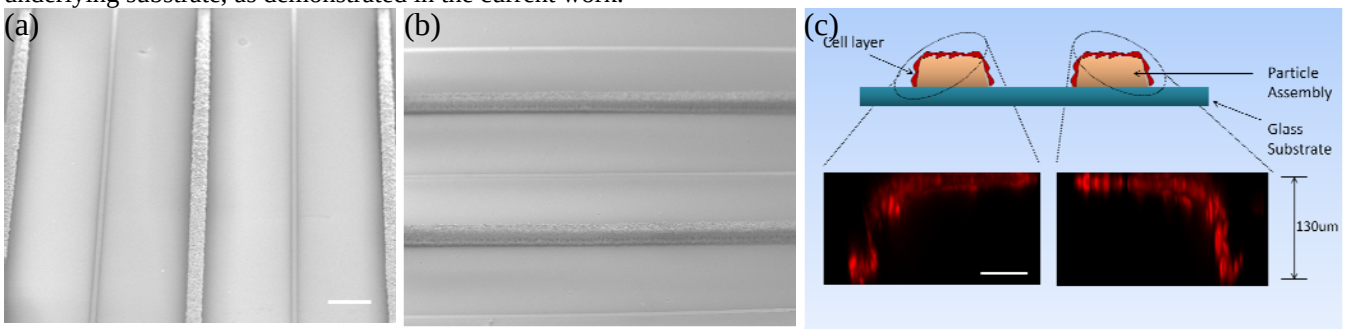

Figure 4: 3D particle organization and 3D cell patterning. (a)Top view SEM; and (b) Side view SEM. Scale bar: 500μm. (c) Fluorescence confocal micrograph showing the cell patterning on curved surface of the particle assemblies. Scale bar: 100μm.

Apart from the high patterning contrast, another benefit of this method is the capability of patterning the particle along the vertical direction into a 3D structure. This was illustrated by the SEM micrographs which show the particle assembly after 3 min electrospray. As shown in Figure 4a&b, particles were well confined in the electrodes regions. The side wall of the particle assembly was essentially vertical with a sharp edge. The cross section showed that the width and the thickness of the particle assembly have the similar magnitudes (data not shown), giving rise to an aspect ratio of about 1.

The patterned 3D structure of microparticles was subsequently used as a cell-growth substrate to mimic *in vivo* extracellular environment. After 72 hr cultivation on BSA modified surfaces, the 3D cell patterning was examined by fluorescent confocal microscopy. The reconstructed confocal images in Figure 4c revealed that cells (denoted by red stain) were able to reside on the sloped sidewall as well as the top surface of the microparticle assembly, which was as high as 130 μm. In contrast, few cells were observed on the planar surface of glass substrate. The curvature and the feature size of 3D substrate are tunable by adjusting both the geometry of electrodes array and the electrospray parameters, which is ideal for mimicking the growing conditions of the endothelial and epithelial cells in their natural environment. Since PCL is degradable, functional bio-system at sub-tissue level such as vasculature endothelial lining and epithelial cell sheets can be easily engineered *in vitro*.

CONCLUSION

This study reports a unique method to fabricate microstructures made up of polymeric particles for 2D and 3D cell patterning. The technological combination of electrospray and microfabrication enables the construction of microporous particulate structures on the cell culturing substrates; while the use of floating electrodes greatly enhances the patterning contrast and leads to a superior 3D patterning capacity. This approach presents a convenient way to pattern living cells on a planar surface as well as on a 3D substratum, which makes it a powerful tool for applications in fundamental cell study, cell-based biosensing and microscale tissue engineering.

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