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• LETTER TO THE EDITOR •

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A simple magnetic force-based cell patterning method using soft lithography

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Dear Editor,

Cell patterning is gaining more and more attention regarding a wide range of applications including cell biology, tissue engineering, and biosensor technology, to name a few. Magnetic force provides a promising tool to pattern cells because it is of excellent biocompatibility, tunable and reversible, and can be employed flexibly in or out of the cell culture system [1]. While extensive studies [2–5] contribute valuable approaches for positioning cells with high resolution, costly and time-consuming fabrication procedures, such as electrodeposition and electrical discharge, still limit the application of these techniques.

In this study, we propose a simple, low-cost magnetic force-based cell patterning method using soft lithography technique. NIH 3T3 fibroblasts labeled with magnetic beads are employed to validate the present method. The cell patterns are well controlled by the microwell pattern on the PDMS substrate fabricated with soft lithography. Furthermore, the variation of cell cluster sizes is investigated and single cell patterning is achieved with high throughput. This technique shows promise as a tool for cell mechanics and tissue engineering research.

The magnetic patterning device (as illustrated in Figure 1A) is composed of a Neodymium disc magnet (surface magnetic induction 380 mT, Beijing Link Technology Co., Ltd.) and a magnetic concentrator fabricated via soft lithography technique. An array of microwells (diameter 50 μm, depth 29.5 μm) are engraved into the PDMS substrate (2 cm×2 cm×0.3 cm, L×W×H). The microwells (100×100 with an interval of 125 μm) are filled with iron powders (AR; Sinopharm Chemical Reagent Beijing Co., Ltd.). The substrate is observed with a digital microscope to make sure the microwells are totally filled and the iron powders between the microwells are completely removed. The microwells are then covered by a uniform PDMS film (21 μm thickness) to separate the iron powders from the cultured cells.

Mouse fibroblast NIH 3T3 cells are cultured in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum (FBS, v/v), penicillin-streptomycin (100 U mL^{-1} penicillin, 100 μg mL^{-1} streptomycin) at 37°C under a humidified atmosphere of 5% CO_2 and 95% air. The NIH 3T3 cells are then prelabeled with carboxylic modified polystyrene magnetic beads (diameter 100–500 nm, BaseLine ChromTech Research Centre) with a concentration of 0.1 ng/cell for 12 h. After the cells are harvested and resuspended with the fresh medium, a magnet is used to accumulate the suspended cells to make sure all the cells seeded on the magnetic concentrator devices are successfully labeled with magnetic beads. With MTT assay, the magnetic beads are proved to have negligible effects on the cell viability (more than 90%, 72 h after labeled).

In order to verify the feasibility of the present method for

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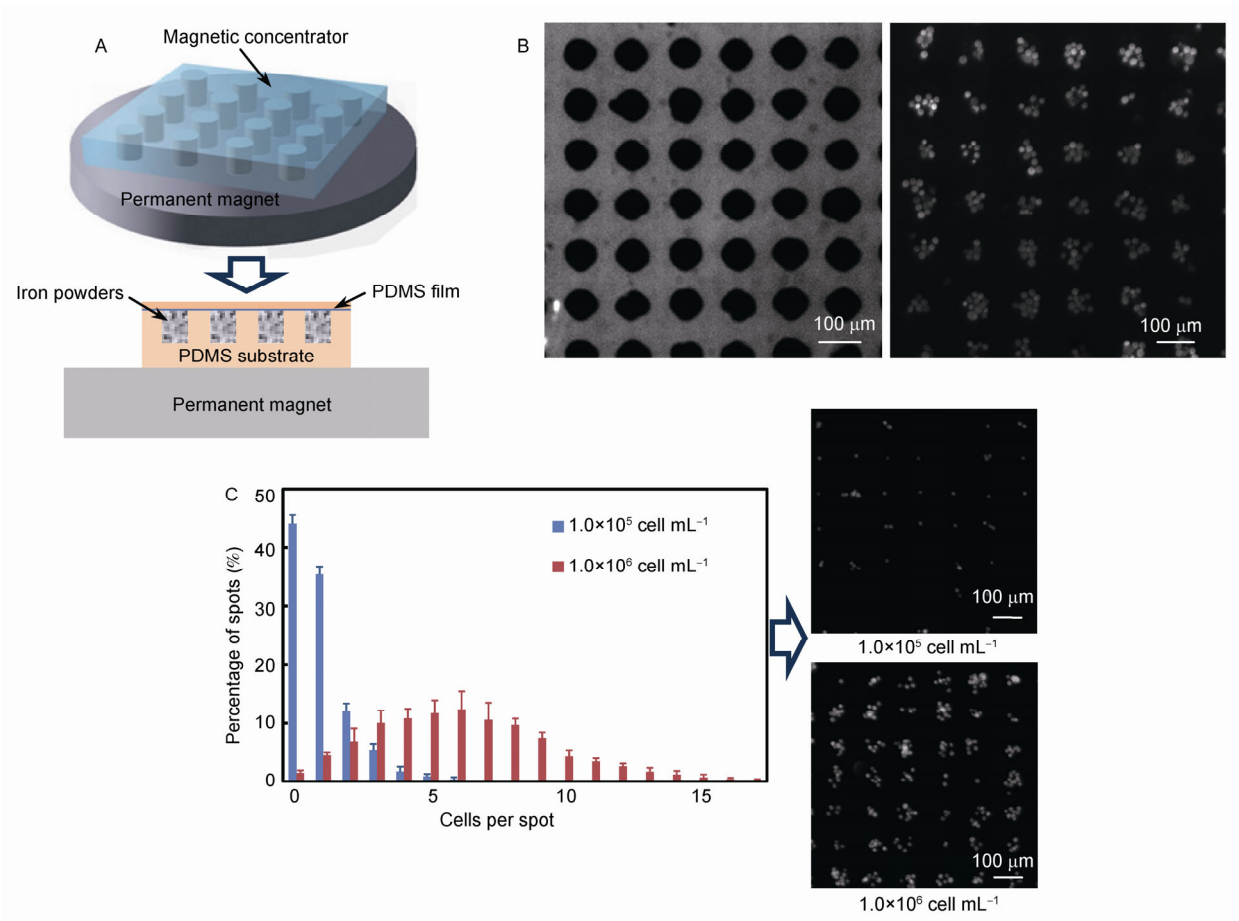

Figure 1 (color online) Magnetic force-based cell patterning method using soft lithography. A, Fabrication of the cell patterning device. B, Generation of cell arrays (fluorescent image, right) with soft-lithography-fabricated microwell patterns (phase-contrast image, left). C, Statistic results of the cell number on each spot in the array using different cell seeding concentrations and the corresponding cell patterns (fluorescent images) on the substrate. The fluorescent images are taken after 2 h cell adhesion.

cell patterning, NIH 3T3 cells labeled with magnetic beads are seeded on the proposed magnetic concentrator and pure PDMS substrate (served as the control group). After 2 h adhesion, the NIH 3T3 cells on the PDMS substrate containing the iron pattern are observed to be positioned and form the exact same patterns as the bottom concentrator patterns (Figure 1B), which demonstrates that the fabricated magnetic concentrator concentrates the magnetic flux of the under magnet and successfully forms cell patterns. The number of the cell cluster in a given area can also be easily controlled by adjusting the horizontal spacing between the microwells in the array. Simultaneously, the cells seeded on the pure PDMS substrate settle randomly since no magnetic concentrator is employed.

The cell cluster size varying with seeding cell densities is shown in Figure 1C. As seeding density increases, the cluster sizes also increase generally. The distribution of cluster sizes approximates a Poisson distribution. This performance indicates that the cells are attracted to each of the spots in an independent fashion, and thus, the cluster size can be controlled by the seeding density. With the increase of the seeding density, the peak of the Poisson distribution shifts to the right and the size of the cell clusters on most of the spots increases from 0–1 cells/spot (1.0×10^5 cell mL^{-1}) to 6–7 cells/spot (1.0×10^6 cell mL^{-1}). With the present technique at a low seeding density (1.0×10^5 cells mL^{-1}), the single cell pattern is achieved (Figure 1C). It is observed that ~36% of the total magnetic spots trap single cells and ~64% of spots with cells are single cell patterns. With single cell patterns, large amounts of cells can be studied in a single experiment while improving throughput and eliminating cell-cell interactions.

In conclusion, cell patterning allows precise control of the geometry, size and location of cell clusters, and thus benefits the study of cell biology, cell-based biosensor techniques, tissue engineering, and so on. In this study, we present a novel, low cost magnetic-based method for cell patterning. The whole device contains only two parts: a permanent magnet and a magnetic concentrator which can be easily fabricated with soft lithography technique. The proposed magnetic concentrator is demonstrated to form cell patterns successfully. Due to the simplicity and low cost,

the present method can be integrated with other macro or micro cell culture systems, such as traditional cell culture dish and microfluidic systems. The good biocompatibility of the label beads also enables the present method to deal with different types of cells (adherent cells and suspension cells). Since the present device can generate high gradient magnetic fields, this characteristic allows for applying the biomechanical stimuli in a high throughput manner (~2500 spots cm^{-2}) at the micro-scale. With these properties, the present method provides a promising technique for cell patterning and biomechanical study at the micro scale.

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