DIRECTED MIGRATION OF CELLS IN CONTACT WITH ANISOTROPIC MICROSTRUCTURES

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ABSTRACT

This paper reports a novel method to orient both the axis and direction of cell migration, using an array of tilted micropillars. . It is well known that chemical or mechanical clues can bias the direction of cell migration. However, very few investigations have been done on the base of anisotropy of geometrical features in cell locomotion control. Here, we show that surfaces covered by tilted micropillars can direct cell motion in different configurations. The versatility and the robustness of this approach make such kind of structures good candidates for both fundamental research and advanced applications, orienting cells in contact with an artificial surface.

KEYWORDS

Cell migration, surface engineering, microstructures, and tilted pillars.

INTRODUCTION

In the past, numerous studies have shown that the inhomogeneity of cell factors, such as chemical or mechanical gradients, can guide cell migration. For example, NIH-3T3 cells on a substrate with a gradient of stiffness are found to move into the stiffer regions [1]. Recently, Mahmud *et al*. showed that the movement cells could be biased by adhesive micro-patterned ratchet [2]. t has been shown that the migrating direction of a neuron can be biased in a ratchet shaped microchannel [3]. In this work, we propose to direct cell migration by placing a surface covered by tilted micro-pillars in contact with the cells. This method is versatile, since it could be applied on any surface in contact with cells, in vivo or in vitro, and does not rely on cells adhering to the surface.

RESULTS

The tilted micropillars shown in figure 1b were fabricated from a mold obtained using tilted lithography (fig. 1a) [4]. Briefly, a layer of photo-resist was spin-coated directly on a copy of a photo-mask and insulated by the backside with a tilted UV light beam. After development, the resist formed a series of tilted pillars which was used to make a mold cavity in PolyDimethylSiloxane (PDMS) (RTV615, GE) by just pouring the liquid polymer on the SU8 structures. After an anti-adhesive treatment of this mold by chemical vapor deposition of trimethylchlorosilane (TMCS) (Sigma Aldrich), it was used to fabricate Sample surfaces including tilted pillars either by soft lithography of PDMS [5], or by embossing Poly(Lactic-co-Glycolic Acid) (PLGA) (Sigma Aldrich) at 90°C, 120 Bar for 10 min.

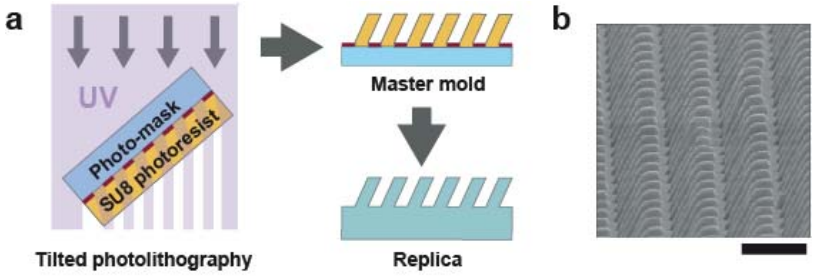

Figure 1: Fabrication of tilted micro-pillars. a) Schematic diagram of the fabrication process. b) SEM image of tilted micro-pillars. Scale bar is 5 µm.

To assess the ability of tilted micropillars in the control of cell migration, we placed them in contact with Normal Human Dermal Fibroblasts (NHDFs) in two different ways (fig.2a-b): i) By using adhesive tilted micro-pillars as a cell culture substrate. In this experiment, pillars were made of PLGA and treated with a fibronectin solution for 30 min (25 µg/ml in PBS) to promote cell adhesion. NHDF cells were seeded directly on the PLGA substrate before observation. ii) By using non-adhesive tilted micro-pillars to confine fibroblasts migrating on a flat adhesive surface. In this case, micro-pillars were made of PolyDimethylSiloxane (PDMS) and treated during 1 hour with Pll-g-PEG (SuSoS, Switzerland) to prevent adhesion of cells (500 µg/ml in HEPES, pH 8.4 after activation of the surface using a plasma cleaner). Additionally, large PDMS spacers of 5 µm height and 440 µm diameter were molded on the flat substrate to control the confinement space of the cells. Before cell seeding, the substrate was incubated in a 50 µg/ml fibronectine solution, allowing cells to adhere. In both experiments, cell were observed in the usual culture medium (DMEM Glutamax+10%FCS+PS, Gibco), DNA of the living cell nuclei was stained with HOECHST (150 ng/ml in the culture medium), and cell motion was recorded for 24 h by fluorescence time-lapse microscopy under controlled atmosphere (37°C, 5% CO_2). Nuclei tracking and migration path statistics were then

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automatically extracted with a custom made software.

Whereas fibroblasts on a standard culture dish follow a persistent random walk [6] leading to an isotropic repartition of the cells around their initial position (fig. 2c left), the cells crawling on the adhesive tilted micro-pillars showed a deviation of migrating direction in the tilt direction of the pillars. (fig. 2c-d center). Interestingly, the migration of cells confined under the non-adhesive structure was also biased in the same direction (fig. 2c right), indicating that the effect is independent of the adhesive properties of the pillars.

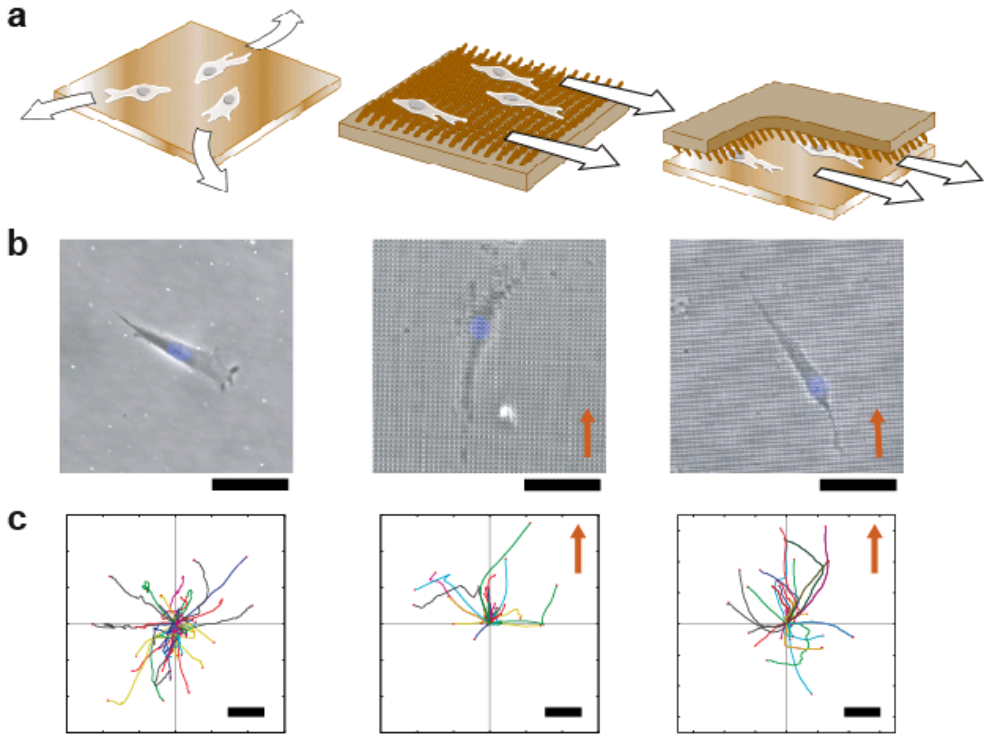

Figure 2: Effect of tilted micro-pillars (5 µm high, spaced by 4 µm, tilted by 30°) on NHDF cells migration. From left to right: Control experiment; migration on adhesive tilted micro-pillars; confined migration under a cover layer with non-adhesive tilted micro-pillars. Red arrows indicate the tilt direction of pillars. a) Schematic diagram of the experiments. b) Typical images of cell (gray is phase, blue is fluorescence of HOECHST). Scale bar is 50 µm. c) Recorded paths of migrating cells. Scale bar is 100 µm.

We quantified the position of the cells relatively to their initial position after 12 h and found that in both cases, most of cells had migrated more toward the tilt direction (fig. 3a). By calculating the distribution of the instantaneous direction of migration on the cell trajectories, we found that, in both cases, cells spent more time migrating in the preferred direction (fig 3b). However, a notable difference between the two configurations could be seen in the recorded cell trajectories: Whereas cells crawling on the adhesive pillars migrated toward the tilt direction from the very beginning (fig 2b center), cells migrating in contact with non-adhesive pillars changed their direction progressively (fig 2b right). This difference in cell motion dynamics suggest two different migration mechanisms. Finally, similar guidance effects toward the tilt direction have been obtained for various geometries of pillars and even of other anisotropic structures like 3D micro-prisms (data not shown), confirming that the geometrical anisotropy of the cell environment has a robust effect on the direction of NHDF migration.

We believe that our observation is helpful to better understand how cell migration is influenced by the geometrical features of the cell environment and that the described phenomenon could be exploited to design new scaffolds for tissue engineering, prostheses surface, wound dressing surface, etc.

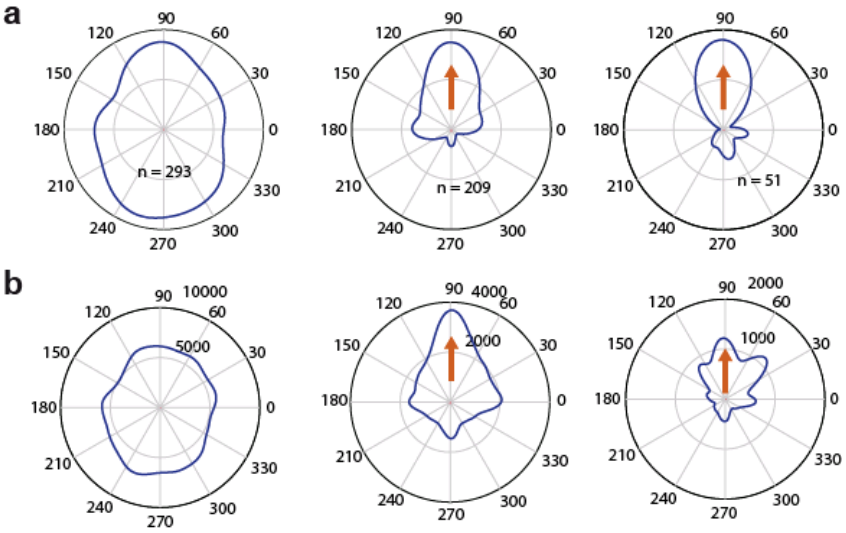

Figure 3: Quantification of the migration bias (same dataset in fig. 2). From left to right: Control experiment; migration on adhesive tilted pillars; migration confined under non-adhesive tilted pillars. Red arrows indicate the tilt direction of pillars. a) Histograms of directions taken by cells after 12 h of migration. n corresponds to the number of measured cells. b) Histogram of instantaneous direction of migration.

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REFERENCES

[1] C.-M. Lo, H. B. Wang, M. Dembo *et al.*, *Cell Movement Is Guided by the Rigidity of the Substrate*, Biophysical Journal, **79**, pp. 144, (2000).

[2] G. Mahmud , C. J. Campbell, K. J. M. Bishop *et al.*, *Directing cell motions on micropatterned ratchets*, Nature Physics, **5**, pp. 606, (2009).

[3] K. Ohnuma, T. Toyota, T. Ariizumi *et al.*, *Directional migration of neuronal PC12 cells in a ratchet wheel shaped microchamber*, Journal of Bioscience and Bioengineering, **108**(1), pp. 76 (2009)

[4] M. Han, W. Lee, S. K. Lee et al., *3D microfabrication with inclined/rotated UV lithography*, Sensors and Actuators a-Physical **111**, pp. 14 (2004).

[5] G. Velve-Casquillas, M. Le Berre, M. Piel, P. T. Tran, *Microfluidic tools for cell biological research*, Nano Today, **5**, pp. 28-47 (2010)

[6] M. H. Gail, C. W. Boone, *The locomotion of mouse fibroblasts in tissue culture*, Biophysical Journal, **10**, pp. 980 (1970).

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