ABSTRACT

Micropost arrays are widely used to quantify cellular traction forces on the substrate; however, the ability to control cell morphology on micropost arrays has remained a challenge. Here we present a biophysical method for inducing cellular alignment on micropost arrays. Experimental results for bovine aortic endothelial cells (BAECs) revealed that comparatively higher interpost spacing along one axis significantly promoted cellular alignment along the perpendicular axis. By the end of 18-hour studies, BAECs were aligned within 26°±4° (n = 39 cells) of the designated axis on average, with 36% of seeded BAECs aligned within 10° of the designated axis. Thus, this methodology could be readily employed to simultaneously achieve both cellular alignment and traction force quantification.

KEYWORDS: Microposts, Micropillars, Cell Alignment, Biophysics

INTRODUCTION

Arrays of microposts (alternatively referred to as micropillars) provide an effective method for quantifying the traction forces of seeded cells [1]. In addition, micropost arrays have been shown to influence a variety of cellular processes, including stem cell lineage specification [2] and directional migration [3]. Methods for controlling the morphology of cells seeded on micropost arrays could further improve researchers’ abilities to direct and study cellular processes; however, current techniques for regulating cell morphology on micropost arrays remain complex. For example, Zhao et al. used a multi-layer fabrication process to embed arrayed microposts between elevated sidewalls and larger posts [4]. To overcome limitations associated with experimental complexity (e.g., cost, time and labor), techniques that utilize biophysical spatial stimuli could offer a promising solution. In prior works, differences in axial spacing between extracellular matrix (ECM) proteins have been shown to influence cellular alignment [5]. Recently, we observed that microtopographic spatial cues can significantly affect directional cell motility [6]. In this work, we vary the axial spacing between microposts to influence the alignment of cells seeded on micropost arrays.

THEORY

Figure 1 shows conceptual illustrations of the micropost array design and working principle. The micropost arrays were designed to investigate the effects of differences in axial micropost spacing on cellular alignment. Specifically, the interpost spacing along the X axis ($I_X$) was increased relative to the interpost spacing along the Y axis ($I_Y$) to promote cellular alignment along the Y axis (i.e., $\theta = \pm 90^\circ$).

Figure 1: Conceptual illustrations of the micropost array design and working principle. The alignment angle ‘$\theta$’ is quantified by the cell’s longest axis deflected from the X axis (i.e. in either the positive or negative direction). By increasing the interpost spacing along the X axis ($I_X$) relative to the interpost spacing along the Y axis ($I_Y$), cellular alignment with the Y axis (i.e., $\theta = \pm 90^\circ$) is promoted.

EXPERIMENTAL

Micropost arrays can be manufactured using a number of high-aspect ratio microfabrication techniques. For this study, micropost arrays were fabricated via standard photolithography and soft lithography methods. Briefly, a 10 µm layer of SU-8 negative photoresist was spin-coated onto a clean Si wafer. Using a photomask, four distinct micropost array patterns were UV exposed onto the layer of photoresist via contact photolithography. The wafer was then developed to become a positive
master for the micromolding process. Next, the silicone elastomer, polydimethylsiloxane (PDMS), was mixed at a 10:1 (base: curing agent) ratio and poured onto the master. After degassing and curing of the PDMS at room temperature (20-25 °C), the elastomer was removed from the master to obtain individual micropost arrays.

Figure 2 shows the fabrication results for the four distinct micropost arrays. Because differences in micropost geometry affect micropost stiffness [1-3], all four arrays included identical microposts of 2.5 µm in radius and 10 µm in height. Additionally, \( I_X \) was held constant at 5 µm for all of the substrates. \( I_Y \) was increased from 5 µm (i.e., for the negative control) to 12.5 µm using increments of 2.5 µm.

Prior to cell seeding, the ECM protein, fibronectin, was selectively microcontact-printed onto the top surfaces of the microposts to improve cell attachment. The substrates were sterilized and submerged in 0.2% Pluronics F127 for one hour to promote attachment onto the microposts. BAEC suspensions were prepared using standard tissue culture techniques, with dilutions in Dulbecco's Modified Eagle Medium (DMEM) media targeting 180 cells/µl. BAECs were manually seeded onto the arrays and incubated for one hour to promote attachment onto the microposts. Substrates with seeded cells were then submerged in CO₂ independent media to displace the DMEM media. Phase contrast microscopic images of the BAECs were taken every 30 minutes for 18 hours in a humidity and temperature controlled environment (37 °C) to generate time-lapse videos. Because cell-cell interactions can affect cellular processes, only BAECs that interacted exclusively with the substrates were used in this study. The software, ImageJ, was used to determine the alignment of seeded cells over the course of the 18-hour studies.

Data was obtained from a total of 99 BAECs. The \( p \) values for this study were calculated via unpaired Student's \( t \) tests. The corresponding two-tailed \( p \) values were determined to assess statistical significance. Differences with a \( p \) value less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Experimental results from the 18-hour time-lapse studies of BAECs seeded on the four micropost arrays are shown in Figure 3. Increasing \( I_Y \) was found to promote cellular alignment with the Y axis. By the end of the studies, BAECs on the \( \theta = 12.5° \) array exhibited an average \( \theta \) of 64°±4° (n = 39 cells), which was significantly higher than both the \( \theta = 7.5° \) array (\( p < 0.05 \)) and the negative control array (\( p < 0.005 \)) (Fig. 3a). Additionally, the percentage of cells aligned within ±30° of the Y axis (i.e., \( \theta = 60° \) to 90°) increased with increasing \( I_Y \) (Fig. 3b). For the \( \theta = 12.5° \) array, 36% of BAECs (14 from a total of 39 cells) were aligned within ±10° of the Y axis (i.e., \( \theta = 80° \) to 90°) by the end of the studies (Fig. 3c).

Figure 3: Experimental results at the end of 18-hour studies for cells seeded on the four micropost arrays. (a) Average cell alignment (i.e., angle ‘\( \theta \)’) on micropost arrays with an interpost spacing along the Y axis (\( I_Y \)) of 5 µm and varying interpost spacing along the X axis (\( I_X \)). Error Bars denote s.e.m.; * and † denote \( p < 0.05 \) and \( p < 0.005 \) statistically significant differences, respectively. (b) Division of cell alignment for each of the four micropost arrays. Dashed line marks 33%. (c) Percentage histogram of cellular alignment on the micropost array with \( I_Y = 5 \) µm and \( I_X = 12.5 \) µm (n = 39).
Cellular alignment over the course of the 18-hour time-lapse studies was also observed. On the “$l_x = 12.5 \, \mu m$" array, cell alignment with the $Y$ axis was found to increase over time (Fig. 4; Fig. 5). Although this trend was revealed for the “$l_x = 12.5 \, \mu m$" array, this response was not observed on the negative control substrate (Fig. 5). Specifically, cells on both substrates initially exhibited an average alignment $\theta$ of approximately 45°, which indicates the absence of axial alignment. On the negative control substrate, cellular alignment did not vary significantly during the studies ($p = 0.21$), as BAECs exhibited a final average $\theta$ of 40°±6° ($n = 22$ cells). In contrast, BAEC alignment on the “$l_x = 12.5 \, \mu m$" array increased significantly over the course of the studies ($p < 0.05$) (Fig. 5).

CONCLUSION

Arrays of microfabricated posts provide a valuable technique for measuring the traction forces of seeded cells; however, regulating the morphology of cells seeded on micropost arrays has remained a challenge. In this work, we demonstrated a biophysical method for inducing cellular alignment on micropost arrays via spatial stimuli. We fabricated four distinct micropost arrays corresponding to differing magnitudes of $l_x$, while $l_y$ was held constant. Experiments with BAECs seeded on the micropost arrays revealed that increasing $l_x$ enhanced cell alignment with the $Y$ axis. For the array with $l_x = 12.5 \, \mu m$, BAECs exhibited an average alignment within 26°±4° ($n = 39$ cells) of the $Y$ axis by the end of 18-hour time-lapse studies. Additionally, cellular alignment was found to increase over the course of the studies. Thus, the presented methodology could offer an effective technique to simultaneously induce cellular alignment and quantify cellular traction forces on the substrate.

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REFERENCES


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