MICROSCALE CONTROL OF MICROPOST STIFFNESS TO INDUCE CELLULAR DUROTAXIS

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ABSTRACT

Cell migration guided by the substrate stiffness, known as durotaxis (a subset of mechanotaxis), has been successfully demonstrated utilizing a micropost array with tunable stiffness (μPAS). Bovine aortic endothelial cells (BAECs) seeded on μPAS substrates displaced an average of 46 μm in the direction of increasing stiffness during 18-hour studies, with 78% of cells exhibiting durotaxis. Furthermore, the magnitude of the stiffness change (Δk) from post to post was found to influence the durotactic response. This new class of passive substrates have wide-ranging implications as many physiological processes rely on directional cell migration.

KEYWORDS: Cell Migration, Durotaxis, Mechanotaxis, Micro-topography

INTRODUCTION

Controlling cell migration is integral to many applications in tissue engineering, biomaterials and medical device implantation. Chemotaxis can be impractical in various conditions and durotaxis offers a promising alternative [1]. Prior durotaxis work has utilized numerous methods to create polymer-based substrates with varying rigidity; however, complicated microfabrication as well as poor control over the placement and magnitude of substrate stiffness are among the limiting factors of these technologies [1, 2]. Thus, advancement of durotaxis-based technologies demands that these limitations be resolved.

THEORY

Previously, arrays of microposts of identical radii (r), and therefore stiffness (k), have been used to quantify cell traction forces (Eq. 1) [3]. Varying post radii significantly changes their stiffnesses, enabling rigidity gradients to be created by tuning geometric parameters (Fig. 1). When BAECs are cultured on μPAS substrates, they spread over multiple posts of varying stiffness and migrate in the designed direction of increasing rigidity.

$$k_{post} = \frac{3\pi E}{4L^3} r^4 \qquad (1)$$

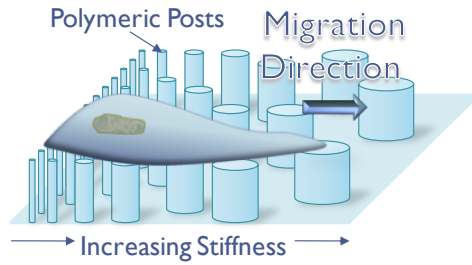

Figure 1. Schematic of the μPAS concept. Micropost stiffness increases in the rightward direction as the radii increase. Living cells migrate rightward via durotaxis.

EXPERIMENTAL

μPAS substrates were fabricated through a one-mask photolithographic process to create a negative master, followed by micromolding of polydimethylsiloxane (PDMS), as shown in Figure 2A. Fibronectin was selectively micro-contact-printed onto the top surface of the posts to improve cell attachment (Fig. 2B).

To prevent haptotaxis-based migratory effects via fibronectin concentrations, micropost spacing was varied to ensure that regimes of different post radii had the same protein density. Micropost radii ranged from 1 to 3.2 μm with identical heights of 7 μm, corresponding to stiffnesses of 0.01 to 1 μN/μm (Fig. 2C).

BAEC migration was studied on two μPAS substrate configurations, differing by their fixed change in stiffness from post to post: 'Δk=.001' and 'Δk=.02' (μN/μm). To quantify migration, cell area centroids were tracked using time-lapse microscopy over the course of 18 hours (Fig. 3). Since cell-cell interactions affect directional migration, data collected from cells with only substrate contact were classified as 'Individual Cell Studies,' while those with additional contact from other cells were classified as 'High Density Cell Studies.'

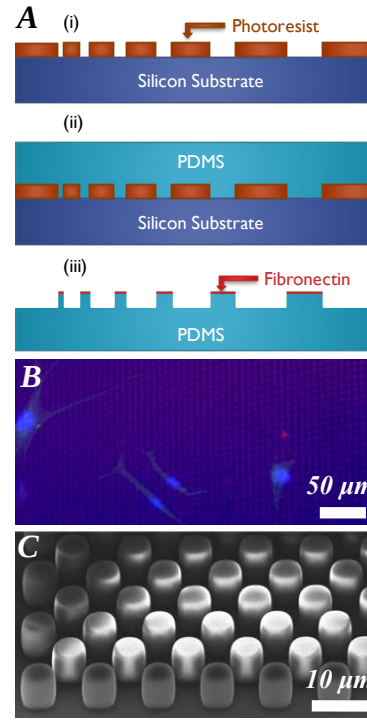

Figure 2. (A) μPAS fabrication process. (B) BAECs on the μPAS under fluorescence microscopy. Fibronectin, actin, and cell nuclei are labeled red (rhodamine), green (FITC), and blue (DAPI), respectively. (C) SEM microphoto of the fabricated μPAS.

RESULTS AND DISCUSSION

Individual cell studies of BAECs seeded on the Δk=.02 μPAS substrates revealed preferential migration toward stiffer microposts (Fig. 4).

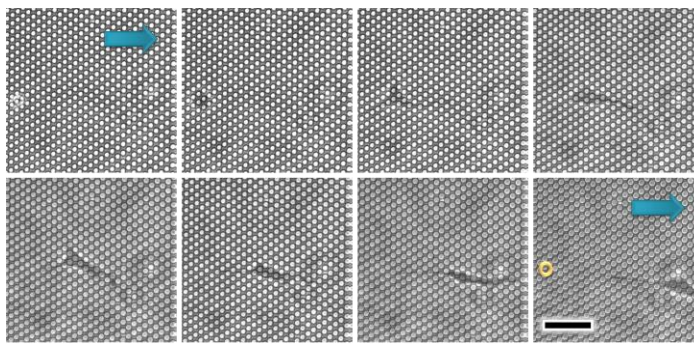

Figure 3. One BAEC migrating on the μPAS over the course of an 18 hour study. The arrows denote the direction of increasing micropost stiffness. The initial position of the BAEC is outlined in yellow for comparison. (Bar=50μm)

On average, the cells displaced 46 μm, with a maximum displacement of 211 μm in the direction of increasing stiffness and 27 μm in the opposite direction. However, when Δk was decreased to .001 μN/μm, the durotactic response was significantly reduced ($p < .05$). In this case, the average displacement of the cells decreased by 73% to 13 μm, with a maximum displacement of 137 μm in the direction of increasing stiffness and 95 μm in the opposite direction. These results demonstrate that the magnitude of Δk can significantly affect BAEC durotaxis.

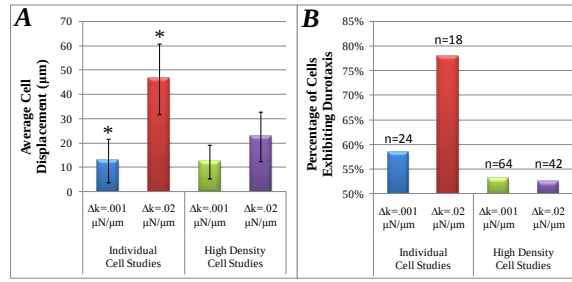

Figure 4. (A) Average BAEC displacement in the direction of increasing stiffness and (B) Percentage of BAECs exhibiting durotaxis on various μPAS substrates. Error bars represent standard error. Asterisks indicate a statistically significant difference ($p < .05$).

Although the cell-cell interactions of the high density cell studies did not greatly influence BAEC migration on the Δk=.001 μPAS substrates, the durotactic response of BAECs on the Δk=.02 μPAS substrates was limited. Here, the average displacement of the cells decreased by over 50% and the percentage of cells exhibiting displacement in the stiffer direction was reduced by 33%. Thus, cell-cell interactions have the potential to greatly affect durotaxis-based migration.

CONCLUSIONS

μPAS substrates provide a simple, repeatable and scalable technique for rigidity-gradient fabrication with high control over the placement and magnitude of substrate stiffnesses. BAECs seeded on the μPAS substrates exhibited durotactic behaviour, migrating in the direction of increasing micropost stiffness. This behaviour was dependent on the magnitude of the post-to-post stiffness change, with higher magnitudes enhancing the durotactic response. Thus, μPAS substrates offer a powerful tool for investigating the cellular response to mechanical properties of the substrate.

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