ABSTRACT
This paper presents unprecedented accomplishments toward unidirectional guidance of cellular migration via durotaxis-based microtopography. In contrast to previous efforts, micropost arrays of varying anisotropy (μPVAs) optimize unidirectional control of cell migration through dual axis durotaxis cues which restrict movement in the lateral direction in addition to promoting migration in the direction of increasing micropost stiffness. Preliminary results show 79% of bovine aortic endothelial cells (BAECs) cultured on μPVA substrates migrated within ±60° of the direction of increasing micropost anisotropy. μPVAs offer a simple, yet powerful technique for enabling unidirectional control of cellular migration for a variety of applications in tissue engineering, biomaterials, and medical device implantation.

KEYWORDS
Durotaxis, Mechanotaxis, Microtopography, Cell Migration, Microposts, Micropillars

INTRODUCTION
Many biological processes, including angiogenesis and wound repair, rely on the directional guidance of cell motility, and it is fundamental for progress in fields such as biomaterials, tissue engineering and regenerative medicine, to achieve high control over cellular migration [1, 2]. Chemical cues have been employed for cellular guidance; however, inherent limitations of such methods have shifted focus to biophysical signals [2]. For instance, certain cell types migrate in the direction of increasing substrate rigidity – a process referred to as durotaxis (a subset of mechanotaxis) [3]. Unfortunately, while durotaxis offers many advantages over other migratory cues, prior durotaxis gradient techniques have shown rather limited results in the control of cell movement due to complicated microfabrication, poor control of substrate stiffness and low repeatability [3-6].

To overcome these limitations, elliptical [5] and variable diameter (circular) [6] micropost array schemes were developed to take advantage of the benefits of microtopography, which include high repeatability, simple fabrication, and high control over micropost stiffness and placement (Fig. 1). However, these arrays were intrinsically limited in controlling cellular migration. Specifically, while elliptical micropost arrays restricted migration bi-directionally along a single axis, unidirectional migration was not achieved [5]. Conversely, variable diameter micropost arrays successfully guided cell migration in a single general direction of increasing micropost stiffness, but movement perpendicular to this direction was not regulated [6].

DESIGN
μPVAs were designed to integrate the advantages of both the elliptical and variable diameter micropost arrays...
to achieve unidirectional cellular migration (Fig. 1). For
multi-axis control, elliptical microposts were arrayed with
increasing anisotropy, where the major axes increase while
the minor axes decrease (Fig. 2). Since micropost stiffness
($k$) is governed by its geometric properties (Fig. 2A), the
durotactic effects of variable anisotropy are two-fold: (i) as
anisotropy increases, the major axis length increases from
post to post, creating a stiffness gradient in this direction,
and (ii) the elliptical shape results in a higher stiffness
along the major axis compared to the minor axis, which
limits lateral migration.

To reduce the potential effects of other migratory cues,
the µPVA was designed to maintain a consistent overall
attachment area, and therefore, extra-cellular matrix
(ECM) protein density. This was accomplished by
adjusting the minor axis length to conserve the percent of
ECM attachment area (%ECM) for posts with differing
major axes (Fig. 2B and 2C). Furthermore, the distance
from post to post along the axis of increasing anisotropy
was held constant to eliminate the potential effects of
variable spacing on migration in this direction. The
microposts had uniform heights of 7 μm, with minor axes
ranging from 2.6-3.0 μm and major axes ranging from 3.0-
5.8 μm, corresponding to physiologically relevant
theoretical stiffnesses of 0.07-0.09 μN/μm and 0.07-0.44
μN/μm, respectively [7].

METHODS
Fabrication
The µPVA substrates were fabricated from the
silicone elastomer, polydimethylsiloxane (PDMS), defined
via a soft lithography process using patterned photoresist
molds. Briefly, clean Si wafers were spin-coated with the
positive photoresist, SPR-220. µPVA patterns were
exposed using 10:1 projection photolithography and then
developed to create negative masters. PDMS (10:1) was
poured onto the masters and degassed. After the PDMS
cured, the finished µPVA substrates were removed from
the wafer. Figure 3 shows SEM microphotos of fabricated
µPVAs. Prior to cell seeding, the microposts were

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\begin{align*}
    k_{\text{major}} &= \frac{3\pi E}{4L^3} (a)(b) \\
    k_{\text{minor}} &= \frac{3\pi E}{4L^3} (a)^3 (b)
\end{align*}
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Figure 2: (A) Schematic of distinct axial stiffnesses, $k_{\text{major}}$ and $k_{\text{minor}}$, for an elliptical micropost. ‘a’, ‘b’, ‘L’ and ‘E’ represent the semimajor axis length, semiminor axis length, micropost height, and Young’s Modulus, respectively. (B) Schematic for the calculation of the %ECM. The top surface area of the micropost ($\pi ab$) is divided by the post-specific surrounding area (WH). (C) Conceptual illustration of the minor axes decreasing while the major axes increase in order to maintain a constant %ECM throughout the µPVA. The actual decrease in the minor axis length over the entire array was approximately 0.4 μm.

Figure 3: SEM microphotos of µPVA microfabrication results. (Left) Expanded view with the direction of increasing anisotropy rightward. (Right) Close-up view of µPVA microposts.
selectively microcontact-printed with the ECM protein, fibronectin, and then treated with Pluronics F127 to reduce cell attachment and protein absorption at locations other than the top surfaces of the microposts.

Experimental

BAECs were seeded on the μPVA substrates for one hour to facilitate attachment and then monitored for 18 hours using phase contrast time-lapse microscopy (Fig. 4). Cell area centroids were tracked to quantify the effects of the μPVA substrates on BAEC migration. As cell-cell interactions can alter migratory behavior, only BAECs that interacted exclusively with the microtopographic substrates and without cell-cell interactions were used for this study.

RESULTS AND DISCUSSION

Studies of BAECs seeded on the μPVA substrates revealed both preferential migration in the direction of increasing anisotropy as well as limited movement perpendicular to that direction (Fig. 5). To quantify the migratory response, directionality was categorized into three regions: (A) migration within ±60° of the designed direction (i.e. the direction of increasing anisotropy), (B) migration within ±60° opposite the designed direction, and (C) migration within ±30° perpendicular to the designed direction. Unidirectional migratory behavior was observed on the μPVA substrates with 11 of 14 cells (79%) migrating into the ‘A’ region by the end of the 18 hour studies, while 2 of 14 cells (14%) migrated opposite the designed direction into the ‘B’ region, and only 1 of 14 cells (7%) migrated into the lateral ‘C’ region (Fig. 5). On average, BAECs displaced 54 μm in the direction of increasing anisotropy by the end of the studies. Compared to BAEC migration on variable diameter micropost arrays, the average magnitude of migration perpendicular to the designed direction for cells on μPVAs was reduced by 19%, corresponding to a 15% decrease in migration to the ‘C’ region. Furthermore, the average cell displacement in the designed direction increased by 16% on μPVAs relative to the variable diameter micropost array studies, corresponding to a 12% increase in migration to the ‘A’ region (Fig. 5).

Although it is clear that BAECs migrated preferentially within ±60° of the direction of increasing anisotropy on the μPVAs (Fig. 5), this result cannot be attributed exclusively to cellular durotaxis for this experiment. In order to maintain a consistent overall ECM protein density, the minor axes of the microposts were decreased slightly as micropost anisotropy increased (Fig. 2). However, because the spacing between microposts along the axis of increasing anisotropy was held constant, larger spacing was required between microposts perpendicular to this axis (Fig. 6). Thus, it is possible that having increased spacing between microposts in the lateral direction (2.0-2.4 μm) versus the spacing in the major axis direction (2.0 μm) may have also contributed to the limited lateral migration. It should be noted that the variable
diameter micropost arrays included much higher lateral spacing relative to the constant major axis spacing. Unfortunately, there are no prior works which describe cellular migration.

Another caveat to this study is that even though the %ECM was held constant throughout the µPVAs, the post-specific top surface area increases from post to post as anisotropy increases (Fig. 2). This was also the case for the variable diameter micropost arrays which actually included much larger increases in the post-specific top surface area from post to post compared to the µPVAs. One solution to eliminate the potential of this mode from affecting migratory results would be to hold the post-specific top surface area constant, in addition to modulating the lateral spacing of microposts in order to maintain a consistent %ECM throughout the array.

CONCLUSIONS
A significant achievement toward unidirectional cellular migration has been demonstrated on µPVA substrates, with 79% of BAECs migrating within ±60° of the direction of increasing micropost anisotropy after 18 hours. By varying micropost anisotropy, two durotaxis-based modes were employed to guide migration in the designed direction of increasing anisotropy while simultaneously limiting lateral migration. µPVA substrates offer a powerful technique for inducing cellular migration by enabling high user control over micropost stiffness and positioning, in addition to the simple and repeatable fabrication process. While further work should concentrate on isolating the durotactic effect, µPVAs show great potential for providing a passive means to achieve unidirectional cellular migration.

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REFERENCES

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