Effects of micropost spacing and stiffness on cell motility

R.D. Sochol[1,2], *A.T. Higa*[1,2], *R.R.R. Janairo*[3,4,*], *S. Li*[3,4,†], *L. Lin*[1,‡]

[1]*Department of Mechanical Engineering, Berkeley Sensor and Actuator Center, University of California, Berkeley, CA, USA*
[2]*668 Sutardja Dai Hall, Berkeley, CA 94720, USA*
[3]*Joint Graduate Group in Bioengineering, University of California, Berkeley & University of California, San Francisco, CA, USA*
[4]*Department of Bioengineering, University of California, Berkeley, USA*
[*]*B104 Stanley Hall, Berkeley, CA 94720, USA*
[†]*B108A Stanley Hall, Berkeley, CA 94720, USA*
[‡]*621E Sutardja Dai Hall, Berkeley, CA 94720, USA*
E-mail: rsochol@gmail.com

Published in Micro & Nano Letters; Received on 14th January 2011; Revised on 4th April 2011

Microfabrication processes enable the biophysical control of cellular environments at the micro- and nanoscale. The mechanical properties of arrayed microposts have been demonstrated to influence diverse cellular functions including cell motility, yet the cellular response to changes in micropost spacing remains unclear. In this work, a microfabricated post array with variable spacing and stiffness was constructed to investigate the effects of these biophysical factors on cell motility. Over a length of 675 μm, the spacing between arrayed microposts decreased from 6 to 2 μm in a single direction, corresponding to an average spacing decrease of 40 nm from post-to-post. Simultaneously, the radii of 7 μm-high microposts were decreased from 2 to 1 μm, resulting in a decrease in micropost stiffness from ~50 to 5 nN/μm, respectively. Over the course of 18 h studies, bovine aortic endothelial cells (BAECs) seeded on the microfabricated post array migrated for an average of 9.6 ± 7.3 μm in the direction of decreasing interpost spacing, opposite the direction of durotaxis. By the end of the studies, 61% of seeded BAECs exhibited displacement in the direction of decreasing interpost spacing. The experimental results suggest that the spacing between microposts can be a determinant factor of cell migration direction in the design of micro- and nanotopographic cellular platforms.

1. Introduction: The capability of controlling the cellular microenvironment is critical for biological applications such as biomaterials, tissue engineering and regenerative medicine [1–3]. Previously, researchers have shown that mechanical cues affect a wide range of cellular processes, including cytoskeletal reorganisation [4, 5], focal adhesion development [6–8] and directional migration (e.g. via durotaxis) [9]. For engineering micro- and nanoscale biophysical systems, micromachining methods offer a variety of benefits, including precise feature definition, simple fabrication and high repeatability [10–13]. For example, microfabricated post arrays have been demonstrated as an effective topographic technology for controlling the stiffness of the substrate because the mechanical properties of microposts are governed by geometric characteristics (i.e. micropost height and radius) [12, 13]. Owing to this property, prior works have employed micropost arrays to quantify cellular traction forces on the substrate [13, 14]. Recently, researchers have geometrically tuned micropost stiffness to influence diverse cellular processes, such as directional migration [15, 16] and stem cell lineage specification [17]. Despite the significant development of micro- and nanotopographic substrates for mechanobiological applications, little is known regarding the impact of micropost spacing on the mechanical response of living cells. In this work, both the spacing between microposts and the radii of microposts are varied over the length of a microfabricated post array to elucidate the effects of simultaneous changes in micropost spacing and stiffness on cell motility.

2. Experimental

2.1. Design: Fig. 1 illustrates the design of the microfabricated post-array. Over the course of the substrate, both the interpost spacing (Fig. 1 – '*I*') and the radii of arrayed microposts (Fig. 1 – '*r*') were decreased from post-to-post in a single direction. All the microposts were maintained at a uniform structural height of 7 μm. As shown in Fig. 2*a*, the interpost spacing was decreased from ~6 to 2 μm over the length of the array, which corresponds to an average spacing decrease of 40 nm from post-to-post. Simultaneously, micropost radii were decreased from 2 to 1 μm.

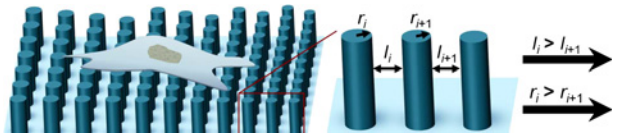

Figure 1 *Microfabricated post array with decreasing interpost spacing (I) and decreasing radii (r) from post-to-post*

Previously, microposts have been modelled as circular cantilevers [13, 14]. Under this assumption, the linear stiffness at the top of the micropost can be approximated as

$$k_{\text{micropost}} = \frac{3\pi\kappa EGr^4}{4\kappa GH^4 + 3EHr^2} \quad (1)$$

where E is the Young's modulus, G is the shear modulus, κ is the shear coefficient, H is the micropost height and r is the micropost radius. As because adjusting the radius of a micropost alters its stiffness, the change in micropost radii over the length of the substrate corresponds to a decrease in micropost stiffness from ~50 to 5 nN/μm, or 0.5 nN/μm from post-to-post (Fig. 2*a*). Thus, the microfabricated post array included mechanical migratory stimuli in the direction of increasing micropost stiffness [15, 16], opposite the direction of decreasing interpost spacing.

Prior works have demonstrated that cell motility can be influenced by substrate-immobilised chemical gradients [18] and variable surface densities of topography [10, 11]. To preclude these effects, the area surrounding individual microposts was modulated to maintain a consistent percentage of extracellular matrix protein coverage (%ECM) and topographic surface density (Fig. 2*b*). Over the course of the array, the %ECM and topographic surface density were held constant at 20%.

2.2. Fabrication: The micropost array was fabricated via standard soft lithography processes as described previously [16]. Briefly, a chrome photomask (Fineline Imaging) was designed with

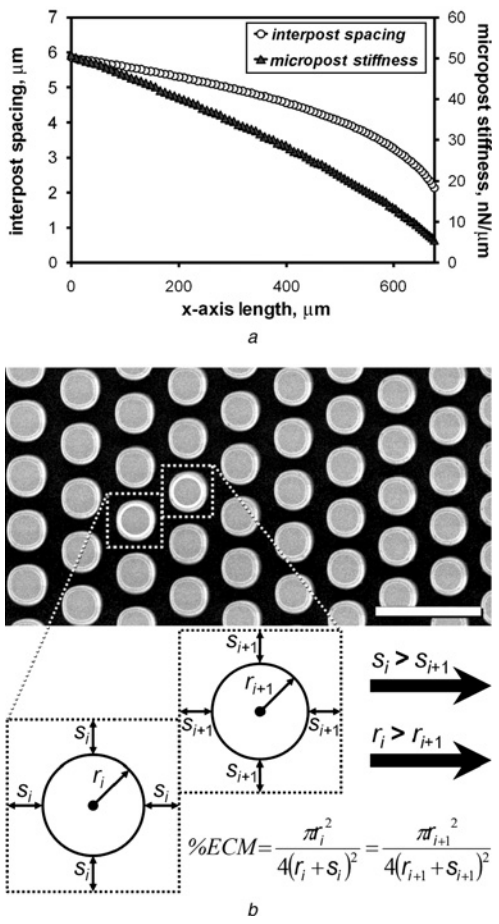

Figure 2 *Microfabricated post array design*
a Interpost spacing and micropost stiffness over the length of the array
b Spacing scheme used to maintain a consistent percentage of extracellular matrix protein coverage (%ECM) and topographic surface density over the course of the array. Scale bar = 10 μm

micropost radii and placement customised as described in Section 2.1. The positive photoresist, SPR-220 (Rohm and Haas Electronic Materials), was spin-coated onto clean Silicon wafers. Microfeatures were photolithographically defined using projection photolithography (GCA-6200 Wafer Stepper, General Signal Corporation). Using the developed photoresist as a negative master, the silicone elastomer, poly(dimethylsiloxane) (PDMS) (Sylgard 184, Dow Corning), was poured onto the wafer at a 10:1 ratio and allowed to cure at room temperature for 2 days. The fabricated micropost array was removed from the negative master. Solvents were not used during the removal process. To remove residual photoresist, the substrate was submerged in 100% ethanol and sonicated for 10 min.

To improve cellular attachment to the top surfaces of the microposts, the substrate was selectively microcontact-printed with the ECM protein, fibronectin (Sigma-Aldrich), via previously described processes [12]. Briefly, the micropost array was treated with O_2 plasma (RTE73 AMNS-500-E, Plasma Therm) for five minutes to render the surface hydrophilic. The surface of a PDMS stamp was incubated with fibronectin (50 μg/ml) for 1 h to allow for protein adsorption. The fibronectin-coated stamp was brought into contact with the micropost array for 15 min to facilitate the adsorption of fibronectin at the tops of the microposts. After the stamp was removed, the microfabricated post array was sterilised and submerged in 0.2% Pluronics F127 (Sigma-Aldrich) for 30 min to limit cell attachment and protein adsorption at locations other than the top surfaces of the microposts. The substrate was stored in sterile phosphate-buffered saline (Sigma-Aldrich) at 37°C.

2.3. Cell motility studies: Bovine aortic endothelial cells (BAECs) were used to study the effects of the microfabricated post array on cell motility. BAEC suspensions were prepared using standard tissue culture techniques, with dilutions in Dulbecco's Modified Eagle Medium (DMEM) media, supplemented with 10% foetal bovine serum (FBS) and 1% penicillin-streptomycin (all obtained from Gibco, Invitrogen) targeting 180 cells/μl. BAECs were manually seeded with ~1 μl of suspension per micropost array and incubated for 1 h prior to recording migration to promote cell attachment onto the microposts. Scanning electron microscopy (SEM) and fluorescence micrographs of BAECs on the microfabricated post array are shown in Figs. 3*a* and *b*, respectively. Substrates with seeded cells were then submerged in CO_2 independent media (Invitrogen) supplemented with 10% FBS to displace the DMEM media, and placed on custom fixtures for enhanced viewing in a temperature- and humidity-controlled chamber that was maintained at 37°C around the stage of the microscope. The recorded cells were not seeded at any particular region of the substrate. Time-lapse videos of cell movement on microfabricated post arrays were generated from phase contrast microscopic images taken over the course of 18 h studies using an Eclipse TE300 inverted microscope (Nikon) with Simple PCI software (Compix, Inc.). The time-lapse videos were analysed using an independently designed cell-tracking programme in conjunction with the software, ImageJ (NIH), to track cell area centroids with time to determine cell displacements and speeds. As cell–cell interactions can affect directional migration [19], data were collected from single cells with only substrate contact. Data were collected for a total of 38 cells from eight distinct experiments. The experimental results are presented as mean ± standard error of the mean (s.e.m.).

3. Results and discussion: Experimental observations revealed that changes in micropost spacing significantly affected the migratory response of seeded BAECs. In prior reports, we observed that on micropost arrays with only mechanical cues (i.e. micropost stiffness increased from 5 to 50 nN/μm with post-to-post stiffness increments of 0.5 nN/μm), BAECs displaced an average of 26.5 ± 8.7 μm in

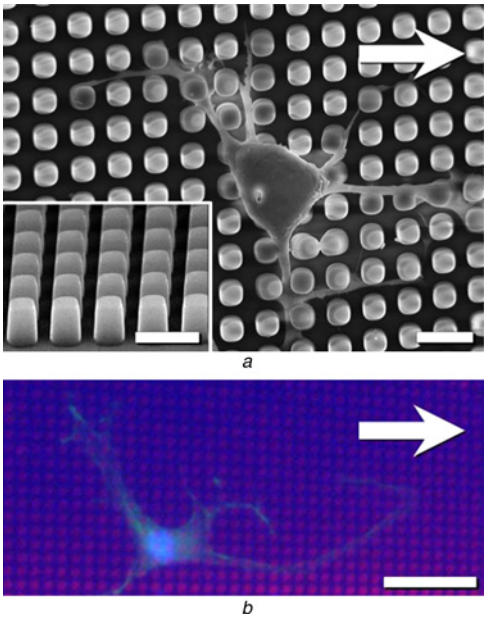

Figure 3 *BAECs on the microfabricated post array*
a SEM micrograph with an enlarged profile view of the microposts (inset). Scale bars = 10 μm
b Fluorescence micrograph. Fibronectin, actin, and cell nucleus are labelled red (rhodamine), green (FITC) and blue (DAPI), respectively. Scale bar = 50 μm
White arrows denote the direction of decreasing interpost spacing and micropost radii

doi: 10.1049/mnl.2011.0020

the direction of increasing stiffness [16]. Here, the addition of variable interpost spacing was found to offset this behaviour. For example, Fig. 4 shows sequential time-lapse images of a BAEC (white arrows) migrating in the direction of decreasing interpost spacing (black arrow), opposite the direction of increasing micropost stiffness. The 2D 18 h cell paths for 38 BAECs seeded on the microfabricated post array are shown in Fig. 5*a*. Although cells were observed to migrate throughout the course of the experiments (Fig. 5*b*), directional biases were not observed initially (Fig. 5*c*). However, by the end of the studies, seeded BAECs were found to displace an average of 9.6 $\pm$ 7.3 μm in the direction of decreasing interpost spacing (Fig. 5*c*). The maximum observed displacements during the studies were 149 μm for cell movement in the direction of decreasing interpost spacing and 115 μm for displacement opposite that direction. In contrast, lateral migration did not appear to be biased, as BAECs exhibited a final average displacement of 4.2 $\pm$ 11.5 μm perpendicular to the axis of decreasing interpost spacing (Fig. 5*d*). At the end of the studies, 61% (23 from a total of 38) of BAECs exhibited displacement in the direction of decreasing interpost spacing relative to their initial positions at the start of the study.

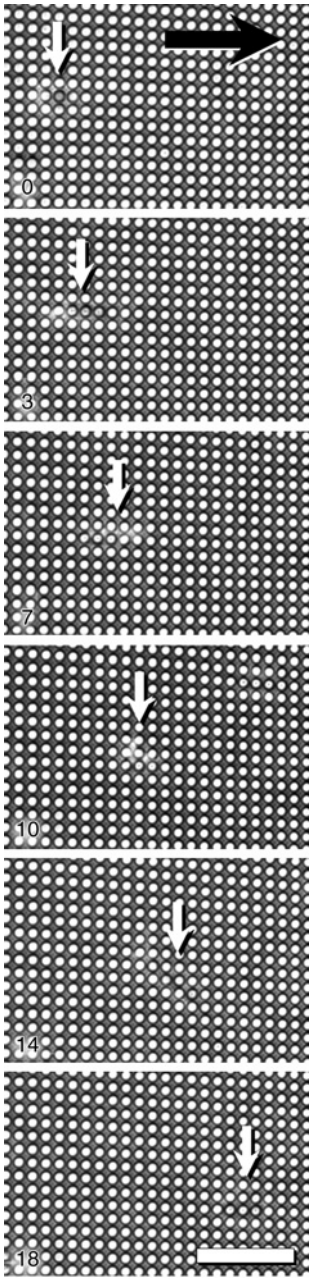

Figure 4 *BAEC migration on the microfabricated post array*
Sequential time-lapse images of a BAEC (white arrows) migrating in the direction of decreasing interpost spacing (black arrow). Units = hours; scale bar = 50 μm

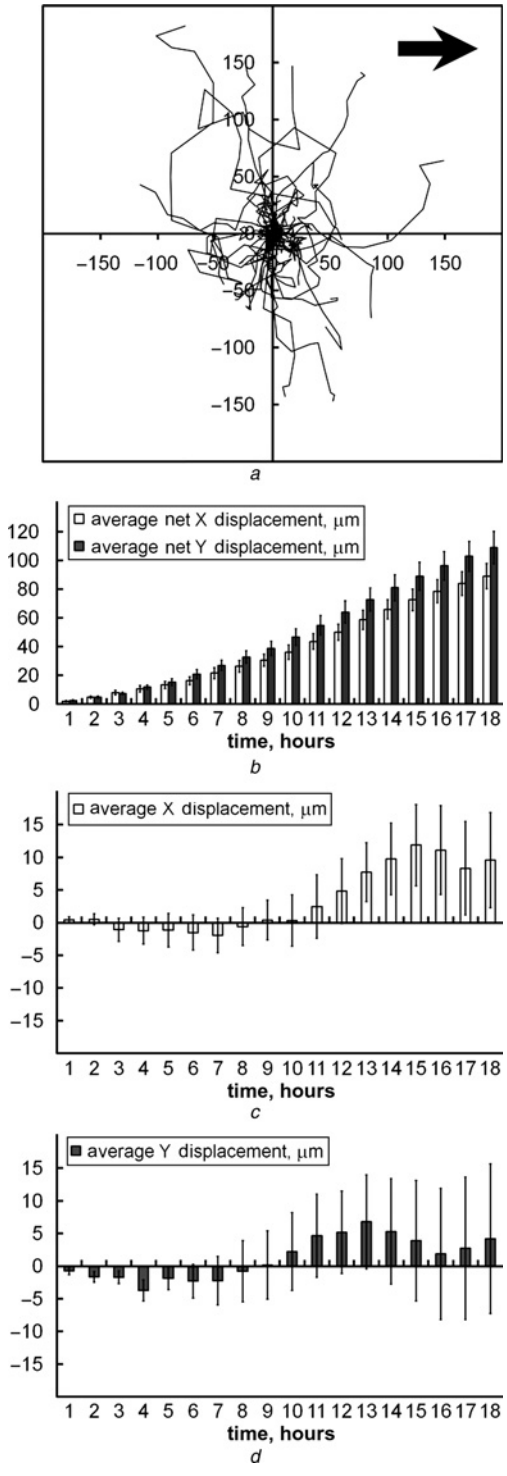

Figure 5 *Experimental results of BAEC migration on the microfabricated post array for 18 h studies*
a 2D cell paths for 38 BAECs. Axis units = μm; black arrow denotes the direction of decreasing interpost spacing
b Average net displacements
c Average displacement in the direction of decreasing interpost spacing
d Average displacement perpendicular to the axis of decreasing interpost spacing
Error bars denote s.e.m.

The speed of cell movement was also found to be influenced by variable micropost spacing. Previously, cells seeded on micropost arrays with only mechanical cues exhibited average speeds of 7.5 ± 0.5 μm/h for movement in the direction of increasing stiffness, and 6.6 ± 0.5 μm/h during migration opposite that direction [16]. The addition of variable interpost spacing appeared to reverse this trend. During the 18 h cell studies, the average speed of BAEC migration was 5.42 ± 0.36 μm/h for movement in the direction of decreasing interpost spacing. In contrast, for migration opposite that direction, BAECs exhibited an average speed of 4.67 ± 0.31 μm/h; however, this difference was not statistically significant ($p = 0.12$).

The experimental results suggest that changes in micropost spacing can limit and even counteract the effects of microtopographic mechanical stimuli. Although the spatial stimuli appeared to be the dominant migratory cue overall, there remained periods where migration was observed opposite the direction of decreasing spacing (Fig. 5*c*). It is possible that this behaviour was caused by the opposing mechanical stimuli. Increasingly the post-to-post spacing increment could further enhance the effects of the spatial cues; however, further study is needed to elucidate the effects of spacing gradient strength on cell motility.

In this work, BAECs were observed to migrate in the direction of decreasing interpost spacing, rather than in the direction of the mechanical cues (i.e. in the direction of increasing micropost stiffness). At present, the specific mechanisms underlying this migratory response remain unclear. One potential basis for this behaviour is that variable interpost spacing may impact the location where the leading cellular protrusion stabilises. During cell movement, the migration direction is dictated by the establishment of a single, dominant protrusion anchored to the substratum; however, protrusive activity is a stochastic and dynamic process [20]. Thus, differences in interpost spacing could result in a higher probability of motile cells first contacting and forming adhesions onto comparatively closer microposts. As a result, cellular protrusions could more readily stabilise in the direction of decreasing interpost spacing, thereby influencing the direction of migration.

4. Conclusions: Micromachining methods are ideally suited for the customisation of cellular environments at the micro- and nanoscale. Microtopographic techniques have previously been utilised to tune the mechanical properties of discrete substrate features to study and regulate cellular functions; however, little is known regarding the role of micropost spacing in these systems. In this work, a microfabricated post array with variable interpost spacing and micropost stiffness was employed to investigate the effects of micropost spacing on the mechanical response of living cells. The motility of BAECs seeded on the microfabricated post array was examined over the course of 18 h time-lapse studies. The experimental results revealed that changes in micropost spacing can significantly limit the effects of mechanical substrate cues on cell motility. In response to post-to-post differences in interpost spacing of 40 nm on average, seeded cells exhibited preferential migration opposite the direction of the mechanical stimuli. By the end of the studies, 61% of seeded BAECs had migrated in the direction of decreasing interpost spacing, with an average displacement of 9.6 ± 7.3 μm in that same direction. As the use of micro- and nanotopographic methods for investigating and controlling diverse cellular processes is expanding, these results suggest that the design of micropost spacing can be critical to the efficacy of cellular platforms for mechanobiological applications.

5. Acknowledgments: The authors thank Dara D. Bahri, Erick Ulin-Avila, Joanne C. Lo, Kedar Shah, Brian Sosnowchik and Terry Johnson for their contributions. This work was partially supported by the National Institute of General Medical Sciences (1 R25 GM56847).

6 References

[1] Mahmud G., Campbell C.J., Bishop K.J.M., *ET AL.*: 'Directing cell motions on micropatterned ratchets', *Nat. Phys.*, 2009, **5**, pp. 606–612

[2] Petrie R.J., Doyle A.D., Yamada K.M.: 'Random versus directionally persistent cell migration', *Nat. Rev. Mol. Cell Biol.*, 2009, **10**, pp. 538–549

[3] Xia N., Thodeti C.K., Hunt T.P., *ET AL.*: 'Directional control of cell motility through focal adhesion positioning and spatial control of Rac activation', *Faseb J.*, 2008, **22**, pp. 1649–1659

[4] Bischofs I.B., Schwarz U.S.: 'Cell organization in soft media due to active mechanosensing', *Proc. Natl. Acad. Sci. USA*, 2003, **100**, pp. 9274–9279

[5] Pelham R.J., Pelham Jr. J.R., Wang Y.L.: 'Cell locomotion and focal adhesions are regulated by the mechanical properties of the substrate – discussion', *Biol. Bull.*, 1998, **194**, pp. 349–350

[6] Galbraith C.G., Yamada K.M., Sheetz M.P.: 'The relationship between force and focal complex development', *J. Cell Biol.*, 2002, **159**, pp. 695–705

[7] Gallant N.D., Garcia A.J.: 'Cell adhesion strengthening and focal adhesion assembly on micropatterned substrates', *Mol. Biol. Cell*, 2004, **15**, pp. 173A–173A

[8] Mack P.J., Kaazempur-Mofrad M.R., Karcher H., *ET AL.*: 'Force-induced focal adhesion translocation: effects of force amplitude and frequency', *Am. J. Physiol. Cell Physiol.*, 2004, **287**, pp. C954–C962

[9] Lo C.-M., Wang H.B., Dembo M., Wang Y.L.: 'Cell movement is guided by the rigidity of the substrate', *Biophys. J.*, 2000, **79**, pp. 144–152

[10] Kim D.-H., Han K., Gupta K., *ET AL.*: 'Mechanosensitivity of fibroblast cell shape and movement to anisotropic substratum topography gradients', *Biomaterials*, 2009, **30**, pp. 5433–5444

[11] Kim D.H., Seo C.-H., Han K., *ET AL.*: 'Guided cell migration on microtextured substrates with variable local density and anisotropy', *Adv. Funct. Mater.*, 2009, **19**, pp. 1579–1586

[12] Sniadecki N.J., Chen C.S.: 'Microfabricated silicone elastomeric post arrays for measuring traction forces of adherent cells', *Methods Cell Biol., Cell Mechan.*, 2007, **83**, pp. 313–328

[13] Tan J.L., Tien J., Pirone D.M., *ET AL.*: 'Cells lying on a bed of microneedles: an approach to isolate mechanical force', *Proc. Natl. Acad. Sci. USA*, 2003, **100**, pp. 1484–1489

[14] Liu Z.J., Tan J.L., Cohen D.M., *ET AL.*: 'Mechanical tugging force regulates the size of cell-cell junctions', *Proc. Natl. Acad. Sci. USA*, 2010, **107**, pp. 9944–9949

[15] Saez A., Ghibaudo M., Buguin A., *ET AL.*: 'Rigidity-driven growth and migration of epithelial cells on microstructured anisotropic substrates', *Proc. Natl. Acad. Sci. USA*, 2007, **104**, pp. 8281–8286

[16] Sochol R.D., Higa A.T., Janairo R.R.R., *ET AL.*: 'Unidirectional mechanical cellular stimuli via micropost array gradients', *Soft Matter*, 2011, accepted for publication, DOI: 10.1039/C1SM05163F

[17] Fu J.P., Wang Y.-K., Yang M.T., *ET AL.*: 'Mechanical regulation of cell function with geometrically modulated elastomeric substrates', *Nat. Methods*, 2010, **7**, pp. 733–795

[18] Hsu S., Thakar R., Liepmann D., Li S.: 'Effects of shear stress on endothelial cell haptotaxis on micropatterned surfaces', *Biochem. Biophys. Res. Commun.*, 2005, **337**, pp. 401–409

[19] Carmona-Fontaine C., Matthews H.K., Kuriyama S., *ET AL.*: 'Contact inhibition of locomotion in vivo controls neural crest directional migration', *Nature*, 2008, **456**, pp. 957–961

[20] Chodniewicz D., Klemke R.L.: 'Guiding cell migration through directed extension and stabilization of pseudopodia', *Exp. Cell Res.*, 2004, **301**, pp. 31–37

doi: 10.1049/mnl.2011.0020