The Development of Patterned Stiffness Substrates for Mechanobiology

W McLain[1], S Snare[1], V Londono, R Superfine

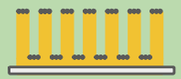
Abstract

Human cell response to has been long studied as cells are responsible for the homeostatic operation of the body. For example, research has detailed that the gradient of rigidity of substrates can lead to cell movement bias, where cells exhibit preference towards the direction of softer, smoother regions. To further understand cell sensing of its mechanical environment, this research investigates the formation of microscale stiffness patterns by patterning two polymers of different stiffnesses. To isolate the effects of stiffness patterns from the potential effects of topography cues, we undertake the challenge of fabricating a flat substrate. To accomplish excellent optical microscopy of the cell response, our substrates need to be optically clear and have a homogenous index of refraction. Finally, substrates must also have high refractive index to allow the technique of total internal reflection microscopy. Topography was verified with fluorescent microscopy, surface homogeneity of the surface with scanning electron microscopy, and stiffness gradients via force mapping. In the future, flat dual substrates could assist in the development of the field of cell motility, allowing for characterization of mechanical cell responses based solely on stiffness patterns. Additionally, protocols developed during this research could allow for future augmentation of the pattern dual substrates used for other purposes.

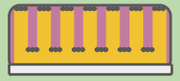
Process Diagram

This is the process of creating fluorescent biosubstrates via fluorescent bead deposition (ranging in size from 40 nm to 1 um diameter)

1. Functionalizing the PDMS array's surface and then depositing s small layer of beads deposited on top of post array Sample



2. The sample is backfilled with Q-Gel (represented in purple). Excess Qgel is bladed off with a straight edge.



A second layer of beads is deposited on top of cured Q-gel

Overview of Experimentation

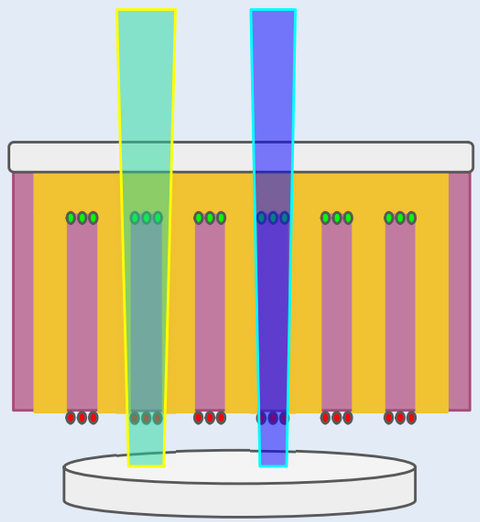


Figure 1. Fluorescent Microscopy Diagram

Fluorescent microscopy used to measure the depth of beads within polymers (biosubstrates) with matching 1.5 indices of refraction, allowing for height analysis

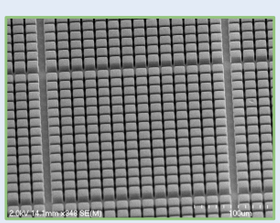


Figure 3. SEM image of Post array

Image shows the posts used in the creation of the OE 50 mold

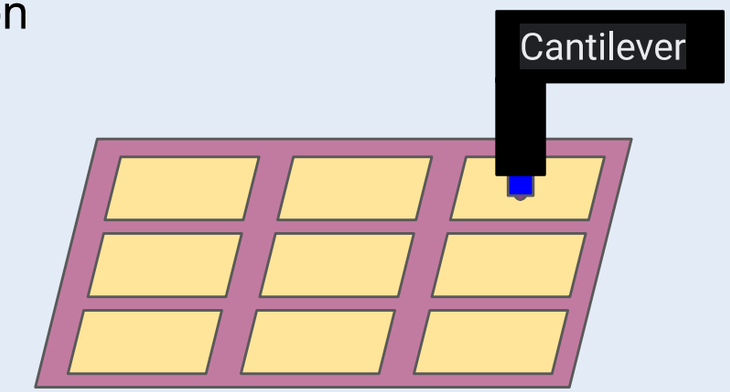


Figure 2. Nanoindentation Diagram

Nanoindentation used in force mapping. OE-50 backfilled with Q-Gel. 19 um diameter spherical cantaliteverantilever used was to create force maps around the stiffness patterned surface

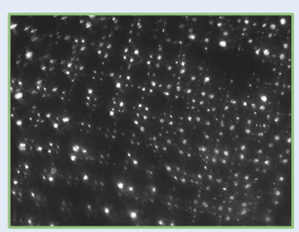


Figure 4. Fluorescent beads on post array

200 nm (Yellow-green) fluorescent beads on post array. Relative height of the sample was measured based on the distance between the red beads and green-yellow beads.

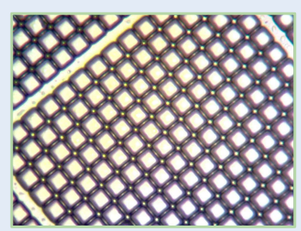


Figure 5. OE-50 mold pre-backfill.

Image details the of the 10x10 um array with a 3 um spacing in between. Additionally, it can be noted that the array has formed holes–acting as the geometric pattern by which the stiffness gradient will be created.

Results

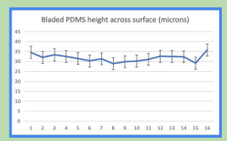


Figure 6. Height of Flat Sample
This details the height analysis performed on a flat sample, which would act as a general target range for these experiments involving the backfilling of a patterned surface.

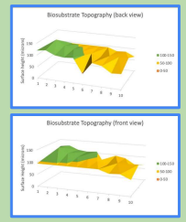


Figure 7. Height of BioSubstrate
The two images above detail the set of height analysis performed (detailing the biosubstrate from two opposite angles). The height analysis details a rough 30 um change in topography across the surface.

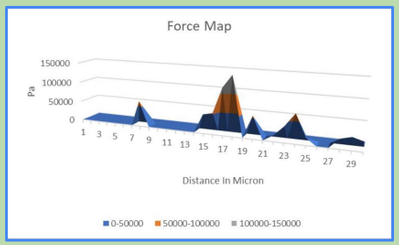


Figure 8. Force Mapping of OE-50
The force map illustrates a 6 x 5 force map of the OE-50 and Q-GEl backfilled biosubstrate. X-intervals between step were 2.4 um and y intervals between step were 20 um.

Discussion

Currently, we have been able to produce dual substrate (backfilled) biosubstrate with a height difference of sub-100 um between the two respective surfaces. This said, the current difference in height and general topography recorded within height analysis could create issue in understanding the effect of stiffness on cells, so future plans are directed at minimizing this height differences by innovating upon current lab practices. To detail, we are currently working to improve our blading (reducing the excess backfilled material on the surface of the patterned substrate)technique-both by practicing the current method and by considering alternate methods of Qgel removal that may be more effective or consistent.