Fabrication of Polymeric Replicas of Cell Surfaces with Nanoscale Resolution

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Introduction

Cells *in vitro* and *in vivo* navigate complex environments through the integration of a wide variety of cues, including diffusible factors, substrate-bound factors, electrical and magnetic fields, substrate rigidity, and topography. Some cues, such as diffusible factors, are easily isolated and have been systematically altered and presented to cells in culture. Other factors, including the topography introduced by one cell type and presented to a second cell type in co-culture, are difficult to introduce without inherently changing a multitude of variables that other cells contribute. So far, the difficulties in reproducing the complex shapes of cells without cellular components has left the influence of cellular topography unexplored. Here, we present a novel method of fabricating transparent, biocompatible polymeric substrates with biomimetic, cell- shaped surfaces with a resolution in the submicron range. As a result, the contribution of cellular topography as an independent factor in co-cultures can be studied for the first time.

Dorsal root ganglia (DRG) have been shown to align to aligned adult rat Schwann Cells (SCs). Here, we utilized SCs grown on microscope glass coverslips as templates for poly-(dimethylsiloxane) (PDMS) replica fabrication and characterized the resulting replicas qualitatively and quantitatively using Scanning Electron Microscopy (SEM) and White-light Interference Microscopy (WIM).

Methods

The fabrication process was divided into 5 stages: (1) production of a polymeric stamp for micro-contact printing; (2) micro-contact printing of laminin stripes onto glass coverslips; (3) cell culture, resulting in rows of aligned SCs; (4) production of polymer I with indented topographical features; and (5) production of polymer II with protruding topographical features.

Initially, PDMS stamps were prepared with repetitive grooves of 60µm width and pitch. Briefly, the pattern was designed in AutoCAD and printed at 10,000 dpi onto a mylar mask. Standard photolithographic techniques were utilized to transfer the pattern onto Si wafers spin-coated with a 50µm layer of negative tone Nano SU-8 50 photoresist, resulting in repetitive grooves with 60µm groove width, 60µm plateau width, and 50µm groove depth. Sylgard 184 PDMS elastomer base was mixed with Sylgard 184 PDMS curing agent at a 10:1 wt/wt ratio, and poured onto a micropatterned wafer to a thickness of 1-2 mm, cured at 95°C for 45 min, and peeled off the wafers to generate substrates of 1cm x 1cm x 1-2mm for micro-contact stamping.

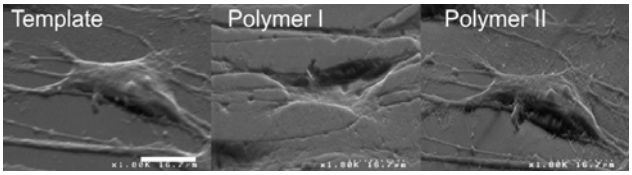
To ensure optimal protein transfer, stamps were submerged in 10% sodium-dodecyl sulfate (SDS) in dH_20, washed, and incubated with 50µg/ml mouse laminin in Hank's Balanced Salt Solution (HBSS) for one hour. Stamps were inverted and contacted glass coverslips overnight that were plasma activated to promote protein transfer, and peeled off, leaving a striped LN pattern.

Adult rat sciatic nerve SCs were cultured from adult rat sciatic nerve. SC were cultured on substrates containing microstamped LN stripes at 90,000 cells/sample in SC media (100ug/mL poly-L-lysine (PLL) in DMEM supplemented with 10%FBS, 1% L-glutamine, 2µM forskolin, 10µg/mL bovine pituitary extract and penicillin (100U/mL)/ streptomycin (100µg/ml) for 3 days.

To allow replica molding, samples were fixed with 1% paraformaldehyde, 2% sucrose in PBS for 15 min at RT, rinsed with PBS, then incubated in Karnovsky's fixative overnight. Samples were post-fixed by with 1% OsO_4 in 0.1M cacodylate buffer for 1h, rinsed with dH_2O, incubated in 0.5% OsO_4 for 30min, dehydrated with graded ethanols, and air-dried.

To generate polymer I, which contains a replica of cellular topographical features indented into its surface, PDMS was applied to the fixed cell sample and cured as before, then removed from the template. To generate polymer II, which contains cellular topographical features protruding from its surface, PDMS was poured onto polymer I, using polymer I as a template, and removed after curing.

To analyze the sizes and orientations of the topographical features at the surfaces of the biomimetic materials we employed SEM and WIM.



SEM micrographs of template, polymer I and II at 1,800x. Scale bar 16.7 um.

Results and Discussion

SEM side-by-side comparisons at magnifications as high as 30,000x and WIM analysis showed that all cellular features, including fine features such as lamellipodia, were accurately reproduced in three dimensions in both polymers I and II with sub-micron accuracy.

In conclusion, we have successfully demonstrated the fabrication of a bio-compatible, transparent, biomimetic surface replica of SCs with a resolution at the nanoscale. These novel biomaterials provide the opportunity to study the isolated effects of cellular topography on cell processes. This technique can be readily adapted to utilize a variety of polymers and to provide replicas for a number of materials including extra-cellular matrix components and whole tissues. The technique can be combined with currently available methods for bulk polymer alteration, surface modification and coating, as well as incorporation of bioactive substances and/or microspheres, allowing environments to be precisely customized in order to study cellular processes.

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