High Fidelity Method for Microfabricating *in vitro* Neural Networks

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Abstract:

Functional *in vitro* models of neural networks are essential for uncovering the underlying cellular mechanisms by which mechanical stress induces traumatic brain injury. However, current models do not capture the connectivity and organization of the brain's axonal tracts, limiting our ability to study how cellular dysfunction may be transmitted between distant regions of the brain. These models are inadequate due to insufficient fabrication techniques for consistently and accurately placing neurons in a network. Here, we propose a novel method for neural network fabrication that incorporates microfluidic cell placement onto micropatterned substrates. We fabricated a microfluidic device featuring cell traps that capture cells in an organized array, placing them in contact with a fibronectin-coated surface that provides guidance cues for network self-organization. As proof of concept, we used 3T3 fibroblasts to assess the efficiency of this technique. Compared to standard microcontact printing, microfluidic delivery of cells resulted in more consistent, uniform adherence of cells to patterned substrates. Development of this technique may allow fabrication of more authentic neural networks and provide a platform by which we may further investigate the role of diffuse axonal injury in the pathophysiological response to traumatic brain injury.

Introduction:

Blast-induced traumatic brain injury (bTBI) is the most frequent injury suffered by American soldiers in Iraq and Afghanistan; however, the mechanism by which blast waves transduce brain injury remains poorly understood [1]. Although *in vivo* studies are the current focus of research, *in vitro* TBI models display the capability of providing unique insight into the cellular mechanisms that underlie bTBI [2].

These studies often involve synthetic biological tissues fabricated via microcontact printing, a technique used to pattern a substrate with extracellular-matrix guidance cues that promote cell and tissue self-organization. However, standard micropatterning techniques cannot consistently fabricate high fidelity neural networks due to lack of control over cell placement on extracellular-matrix features.

Here, we present a method for precise alignment of cells on a micropatterned substrate using a microfluidic device. Our device isolates and immobilizes cells in a uniform array, allowing for the adhesion of single cells to a micropatterned substrate with precise control over their relative positions. We utilized this technique to seed 3T3 fibroblasts on a network pattern of fibronectin, resulting in consistent cell connections resembling that of neural circuits.

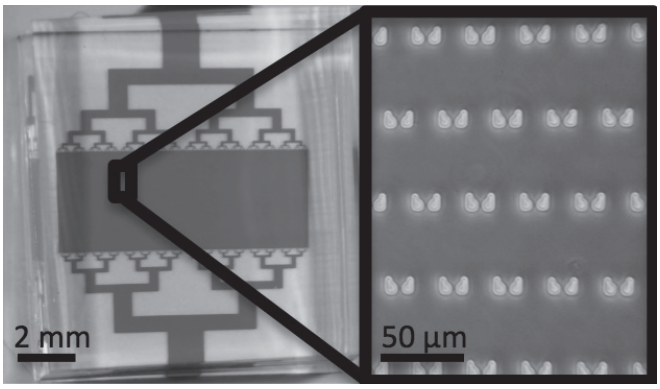

Figure 1: Microfluidic device featuring cell traps to immobilize cells.

Experimental Procedure:

A polydimethylsiloxane (PDMS) microfluidic device (Figure 1) was designed in reference to a device used in [3]. Masters were fabricated from SU-8 3025 photoresist spun on silicon wafers using standard photolithography techniques. Masters were coated with Sylgard 184 PDMS (10:1 base:curing agent), degassed, and baked at 90°C for four hours.

A neural network pattern shown to most effectively promote polarized neural connections was adapted from [4]. PDMS stamps for this pattern were fabricated similarly to the microfluidic device. Stamps were coated with fibronectin (50 mg/mL in H_2O) and incubated for one hour. Fibronectin was transferred to PDMS-coated glass coverslips following the procedure in [5].

A microfluidic device was aligned and temporarily sealed to a micropatterned coverslip using visual cues added to stamping patterns. Channels were rinsed with phosphate-buffered saline (PBS). A 3T3 fibroblast suspension (100 μL; 120,000 cells/mL) was drawn through the device using a house vacuum.

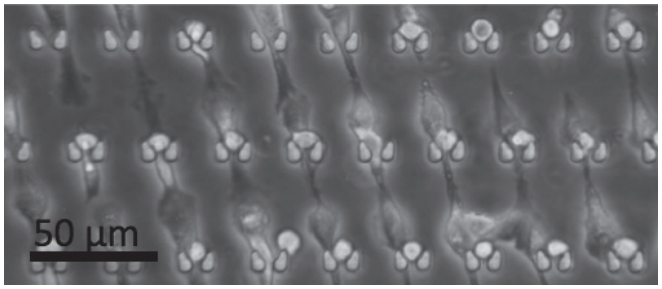

Figure 2: Cells seeded on a network pattern after two hours of incubation.

Immobilized cells were rinsed with culture media and incubated for two hours at 37°C and 5% CO_2 to allow network formation (Figure 2), followed by device removal.

Cells were fixed and permeabilized with 4% paraformaldehyde and 0.05% Triton-100 respectively (in PBS at 37°C). Cells were stained with AlexaFluor 488 Phalloidin and 4',6-diamidino-2-phenylindole (DAPI) in PBS, and imaged using an Olympus IX81ZDC inverted confocal microscope.

Results and Discussion:

To assess the accuracy of cell transfer, fibroblasts were seeded on uniform fibronectin using microfluidic delivery. Adhered cells retained their orientation while immobilized in the capture cups, resulting in a highly organized array of cells compared to that obtained using standard techniques. The radial distribution function of fluorescently labeled nuclei displays areas of high cell density corresponding to known distances between capture cups (Figure 3), confirming the ability of this technique to influence the relative position of cells adhered to a substrate.

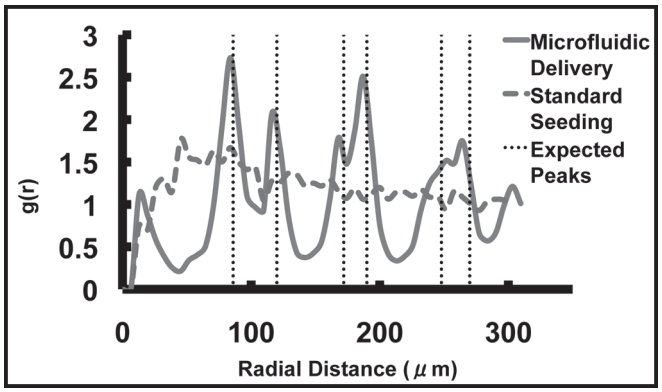

Figure 3: A radial distribution function of fluorescently labeled nuclei.

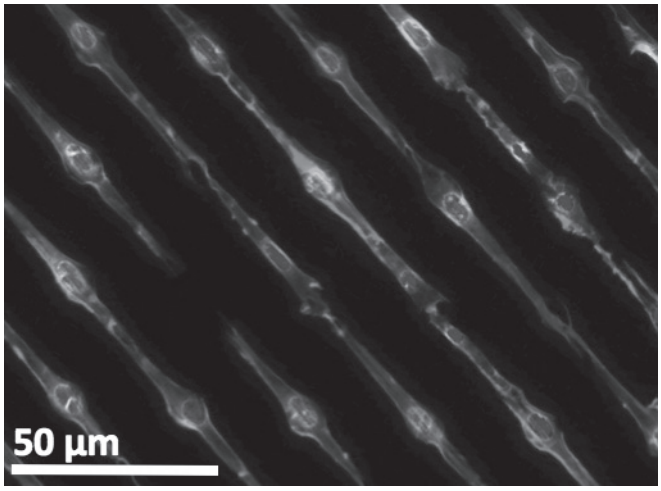

Figure 4: Fibroblasts seeded on a network pattern of fibronectin.

To investigate a potential application of this technique, fibroblasts were seeded on a fibronectin pattern resembling a neural network. Cells were accurately delivered to circular features, and extended radially to form physical contacts with neighboring cells (Figure 4). The linear nature of fibroblast nuclei affirms the success of this technique. When we extend this technique to primary neuronal cell lines, accurate soma placement may promote polarized neural connections and therefore a functional network.

Improved alignment of cells on a substrate as shown here can benefit tissue fabrication beyond neural systems. Countless distributions of cells, and therefore tissue models, can be obtained from adjusting the array of cell traps. For example, endothelial and smooth muscle cells captured in an array of concentric circles could be transferred to ring of extracellular-matrix to form structures resembling arterial cross sections. Models such as these could be employed in studies of vasospasm and other diseases characterized by blood-vessel hypercontractility such as bTBI.

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