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A Microfluidic Approach to Stiffness Gradient Generation in Polyacrylamide-Based Cell Migration Analysis Platforms

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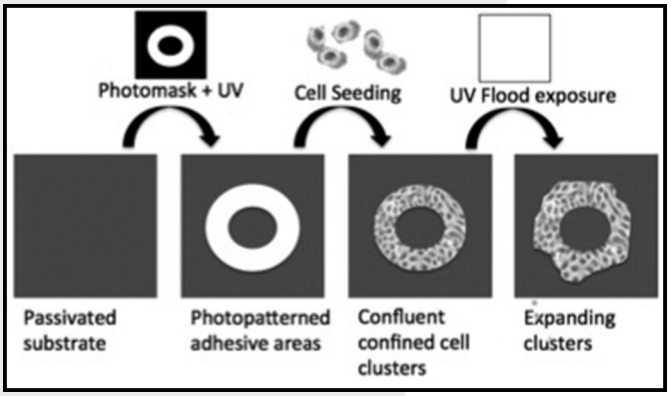

Figure 1: Passivation of substrate with PCP (2k, 5k 12h later) makes the surface nonadhesive. UV exposure of PCP-functionalized surface cleaves the PEG molecule, making exposed surfaces (photomask controlled) cell adhesive. Geometric confinement is determined by irradiation pattern. Cell spreading is initiated by non-selectively exposing the surface following cell seeding.

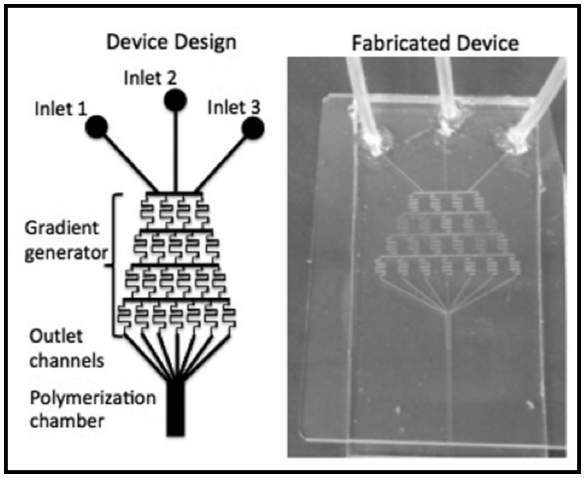

Figure 2: Device is designed for addition of 4% acrylamide in each inlet, and 0.4% bis-acrylamide in Inlets 1 and 2, and 0.04% in 3. For gradient characterization, 20 mM fluorescein was added to Inlet 1, 10 mM to 2 and milliQ H_2O to 3.

Introduction:

Collective cell migration is a critical component of physiological and pathological processes. This motility is directed by extracellular matrix (ECM) factors, including elasticity, known to profoundly affect single cell migration [1]. Less studied are the effects of mechanical compliance on collective cell migration, in which cell-cell contacts are maintained. Understanding the roles of ECM factors in collective cell migration will reveal underlying mechanisms of wound-healing, developmental, and metastatic processes [2].

Until recently, cell migration was studied on stiffness-homogenous substrates, limited in the neglect of durotaxis' stiffness gradient-directed migration [1, 3]. Gradients in niche elasticity often result from the pathological and physiological conditions involving collective cell migration, suggesting that gradients are crucial to directed colony migration.

Microfluidic gradient generation fabricates a more-appropriate substrate for comprehensive motility study, with a precise, function-defined gradient [4].The gradient substrate is achieved by altering polyacrylamide (PAA) crosslinking density and photopolymerizing within microchannels [5, 6].

A controlled collective motility assay may be performed with surface functionalization via photo-cleavable poly(ethylene glycol) (PCP), to direct initial colony configuration and migration initiation [7, 8]. This method has successfully demonstrated collective migration trends in defined micro-environments.

The techniques of substrate formation and functionalization in this study may result in platforms with physiologically-relevant stiffness gradients and capability for light-driven alteration of cell adhesion for sophisticated motility analysis. With the fabricated device and proposed application, collective cell migration is explored to better mimic relevant pathways *in vitro*, and regulate pathways *in vivo*.

Experimental Methods:

Device Fabrication and Construction. The microfluidic device incorporated tri-inlet features, a linear gradient generator [4-5], and a gel photopolymerization chamber (Figure 2). The device was fabricated in PDMS using rapid

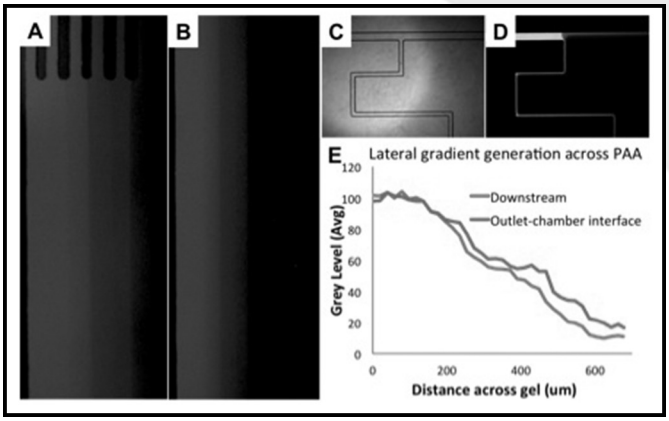

Figure 3: Fluorescent images are of gradient distribution of fluorescein across chamber width at the outlet-chamber interface (A), and downstream (B). Intensity as a function of width indicates a stepwise trend at the interface, and a linear gradient downstream (E). Adequate mixing is achieved at interfaces (C-D).

prototyping and soft lithography [9]. The PDMS component with embedded microchannels and 1.3 mm inlets, was bonded to a glass slide via O_2 plasma treatment (150 mTorr, 100 W, 1 min). Tubing (2 mm) interfaced with the inlet holes via silicon adhesive and connected to a syringe pump.

Device Characterization. Uranine fluorescent dye (MW = 332 Da) was utilized to verify gradient linearity (Figures 2-3). Fluorescent images were obtained during flow, 10-15 min after gradient establishment. Fluorescent intensities were plotted as a function of chamber width using Metamorph (Molecular Devices, CA).

Cell Micropatterning on Bulk Substrates. PAA substrates were photopolymerized on glass slides at 55 and 5 kPa [10]. Compliance measurements were performed via atomic force microscopy (AFM) and a steel bead indentation method [11]. The photopolymerization process was later modified to include methylene blue as the photoinitiator [6]. Surface functionalization via PDL and PCP was performed [7], and a patterned photomask was used in irradiation of adhesion geometries (Figures 1 and 4).

Results and Future Work:

Multiple gradient-generating devices were successfully fabricated with differing outlet dimensions approximating the design parameters. Plots of fluorescence against chamber width at the outlet-chamber interface, and downstream in chamber indicate stepwise and linear gradients, respectively (Figure 3). Thus, the substrate will be extracted at the downstream location.

Before focusing on a gradient gel study, homogeneous gels were successfully fabricated at stiffnesses of 55 kPa and 5 kPa. Stiffness measurements collected via AFM and classical measurements were accurate and comparable. Results of bulk substrate surface functionalization correspond with previous studies [7, 8]. Irradiated regions of passivated substrate had a significantly-greater cell adhesion than non-irradiated regions (Figure 4).

Given the success of bulk substrate fabrication, characterization, and micropatterning, and substrate gradient verification with the fabricated device, we intend to proceed to fabricate stiffness-variant substrates within the device for extraction and migration study. Gradient-compliant substrate photopolymerization is proposed as described in Zaari, et al., with methylene blue [5-6].

Following substrate extraction, techniques of surface functionalization via PDL and PCP should facilitate cell micropatterning and controlled migration initiation [7-8]. The performance of this assay will be the first study of micro-controlled collective migration on a stiffness-gradient substrate with high precision. The information yielded in studies utilizing substrates fabricated with our device will well-define the role of elasticity gradients in collective migration, contributing to mimicry, alteration and understanding of biological processes.

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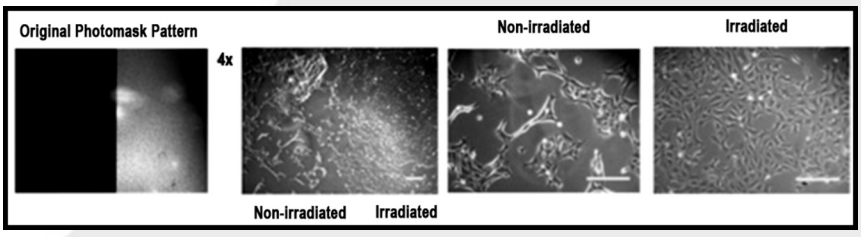

Figure 4: Selective cell patterning was demonstrated on gels with a bulk stiffness to verify process. Cell confluency corresponds with original photomask pattern, where non-irradiated surfaces demonstrate less cell adhesion.