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-functionalyzed 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$_2$O 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 [4,5]. 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
prototyping and soft lithography [9]. The PDMS component with embedded microchannels and 1.3 mm inlets, was bonded to a glass slide via O₂ 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 microcontrolled 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.

**Acknowledgements:**

The author would like to thank the National Nanotechnology Infrastructure Network International Research Experience for Undergraduates Program, National Science Foundation (NSF), National Institute for Materials Science (NIMS), Dr. Jun Nakanishi, Dr. Yoshihisa Shimizu, Dr. Tomonobu Nakayama, Dr. Noni Creasey, Tomoko Ohki, and Akihiko Ohi.

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