High-Throughput Method for Microfluidic Placement of Cells in Micropatterned Tissues

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Project and Objectives

Abstract

Recent studies have shown that cell shape and tissue structure can dictate functional behavior in engineered tissues[1]. One method for controlling tissue structure *in vitro* is microcontact printing, where extracellular matrix proteins are patterned on a substrate to construct arrays of single cells or multicellular tissues. This technique is used to create tissues that mimic *in vivo* architecture which can be used to study tissue properties and disease mechanisms [2]. Traditional seeding of cells on the substrate is imprecise, but our group has developed a microfluidic device for spatial control of cell seeding, which creates more replicable high-fidelity tissues. However, the current method is low-throughput and labor intensive. Here, we present a scalable system of multiple microfluidic devices for parallel cell seeding. This high-throughput, precise approach reduces experimental variation, making biochemical assays on single cell arrays possible in future work. We will use this system to create large arrays of single cells of various shapes for phenotypic studies and to create arrays of tissues with varying cellular organization.

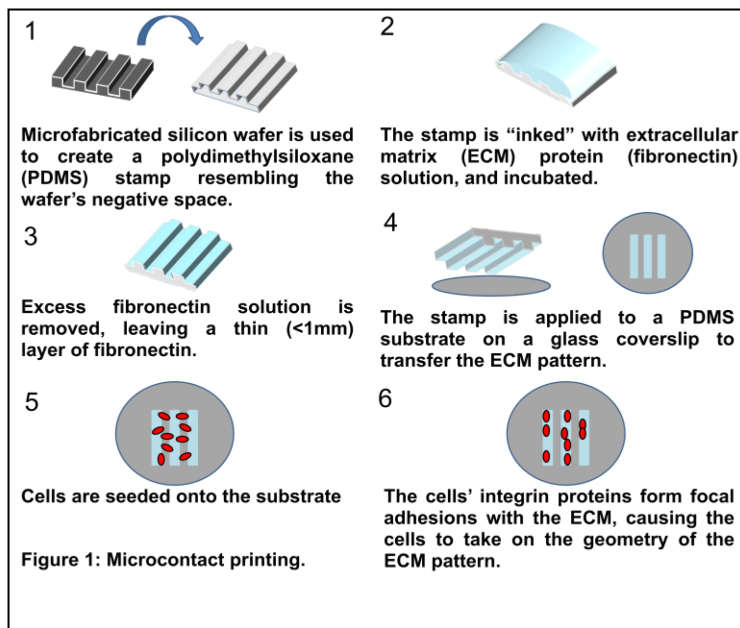

Figure 1: Microcontact printing

Methods

In microcontact printing (Figure 1), the uncured polymer polydimethyl siloxane (PDMS) is poured onto a silicon wafer to create a PDMS stamp. When the cured PDMS is peeled away from the wafer, it has micron-scale features that match the geometry of the wafer's

negative space. The PDMS stamp is then inked with the extracellular matrix (ECM) protein fibronectin. The excess fibronectin solution is removed so that a thin layer remains on the stamp

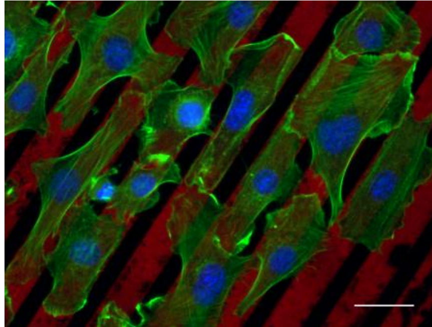

Figure 2: Fluorescent image of tissue generated by microcontact printing procedure with 3T3 fibroblasts. Red: extracellular matrix protein (fibronectin) lines 20 µm in width and 5 µm apart. Blue: DAPI (nuclei). Green: Phalloidin (F-actin filaments). Scale bar= 30µm.

and then the stamp is applied to a PDMS substrate on a glass coverslip to transfer the pattern. Cells are seeded onto the substrate, and the cells' integrin proteins form focal adhesions with the ECM pattern, so the cells take on the geometry of the stamped pattern. For example, in Figure 2, the cells can be seen taking on the elongated geometry of the line pattern.

To use the microfluidic device with this procedure (Figure 3), the device is applied to the substrate after the patttern has been stamped. Cell suspension is applied at the inlet and a vacuum is applied at the outlet to flow the cell suspension through the device. The device contains traps for catching cells and support pillars the cells flow around. The cells also flow around filled traps. After flowing, the cells are allowed to attach to the substrate and the device is removed, leaving a very

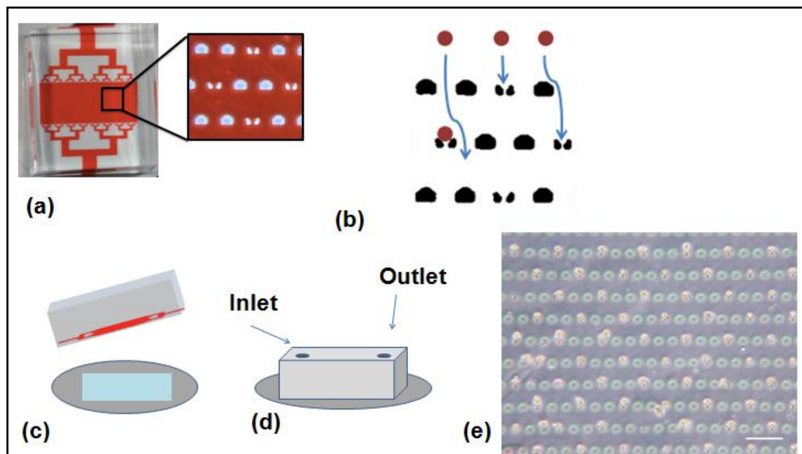

Figure 3: Microfluidic seeding device.
(a)The microfluidic device contains traps for catching cells as they flow through, and pillars the cells flow around[2].
(b) Schematic cell paths through a section of the device.

(i) Cell approaches a filled trap and flows around it.
(ii) Cell approaches an empty trap and is caught in it.
(iii) Cell approaches a pillar and flows around it.
(c) The device is applied to the inked substrate[2].
(d) Cell suspension is introduced at the inlet and a vacuum is applied at the outlet to draw the suspension through the device. After seeding, the device is removed from the substrate (not shown).
(e) Cells trapped in the device. Scale bar=100µm

ordered array of cells on the pattern.

For this project, single devices, devices in parallel as shown in the "System Design" section, and traditional random seeding were used on uniform fibronectin patterns. The cells were imaged after being fixed and having their nuclei stained with DAPI. MATLAB codw was used to perform analysis and generate a radial distribution function to quantify cell alignment.

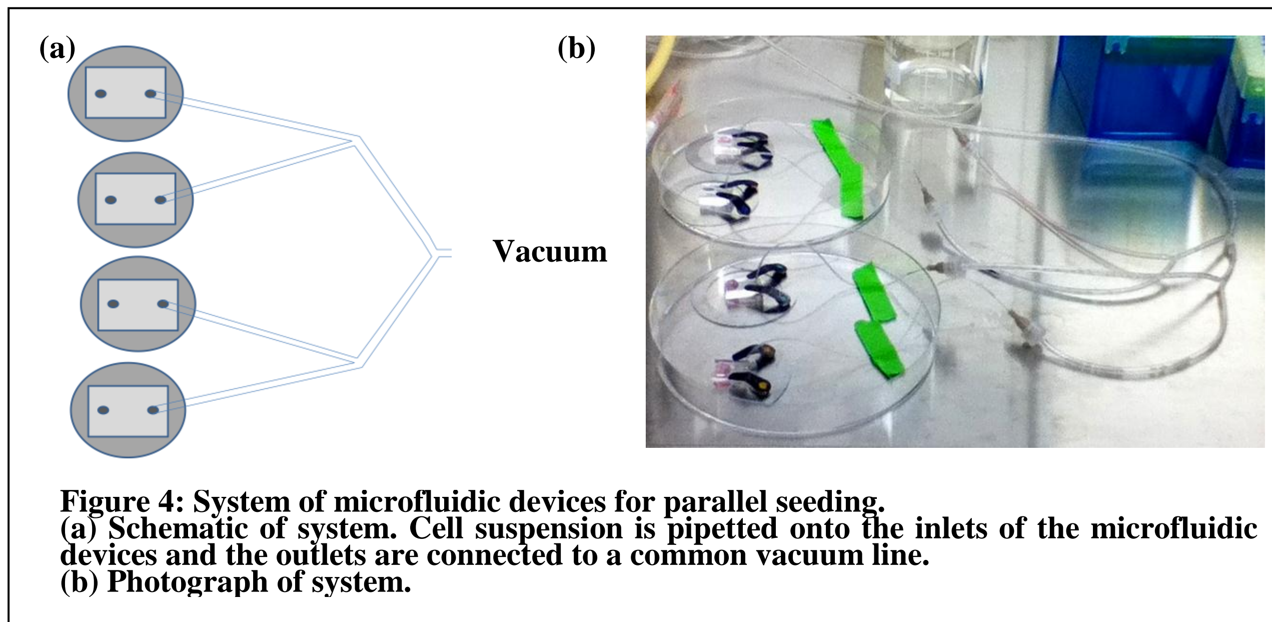

Figure 4: System of microfluidic devices for parallel seeding.
(a) Schematic of system. Cell suspension is pipetted onto the inlets of the microfluidic devices and the outlets are connected to a common vacuum line.
(b) Photograph of system.

System Design

I designed a system and procedure to use multiple devices simultaneously (Figure 4). Similar to using a single device, the cell suspension is pippetted onto the inlet. This allows for the best visibility: the experimenter can see the suspension and when it is getting low. The risk of air bubbles in the device from aspirating air is decreased. Small diameter tubing from the outlet is connected to larger diameter tubing that is arranged with symmetrical bifurcations to connect to the common vacuum line, which allows for even flow generation. Supporting the devices was a major consideration also, this was done using magnets and metal rings. The devices and coverslips were placed in two petri dishes for experiments so that the magnets would not interact.

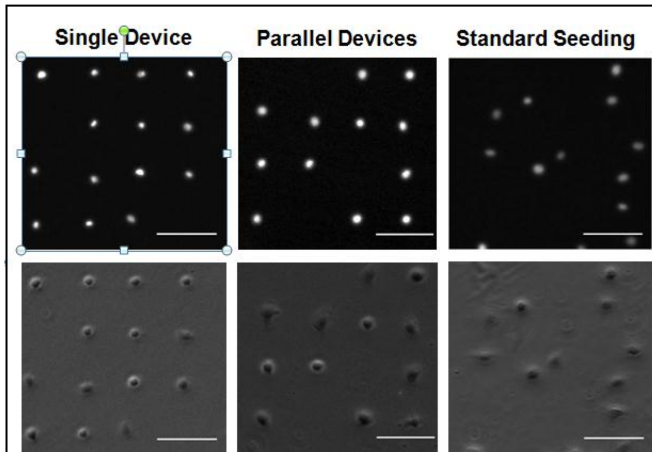

Figure 5: 3T3 fibroblasts seeded onto uniform fibronectin pattern.
Top Row: Fluorescently stained nuclei
Bottom Row: phase contrast images of cells
All scale bars are 100µm

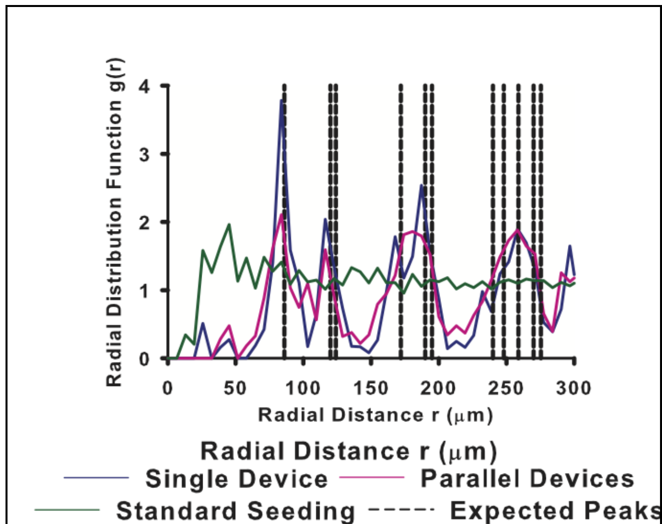

Figure 6: Radial distribution compares the spatial organization of nuclei of 3T3 cells seeded on uniform fibronectin. The ordered arrangement of cells produced by the microfluidic device gives a characteristic radial distribution function with specific expected peaks. We show that using the system of devices reproduces this result, meaning that the seeding with the system has the same precision as seeding with single devices.

Results and Discussion

Qualitative and quantitative results show that using the system to run devices is as precise as using a single device. Images of fluorescently stained nuclei from both single-device and multiple device experiments show an ordered arrangement (Figure 5), while images from a traditional random seeding experiment do not. We quantify this with a radial distribution function (Figure 6), which measures the likelihood of finding another cell a given distance from a cell. A radial distribution function value greater than one means there is greater than random chance of finding a cell at that distance; a value less than one corresponds to less than random chance. Because of the ordered arrangement of cell traps in the microfluidic device, there are characteristic distances between trapped cells, which correspond to expected peaks on the radial distribution function.. The radial distribution function confirms

quantitatively that the system is as precise as single device seeding, and the objectives of this project were effectively accomplished.

Controlled cell placement and simultaneous seeding enable cells to experience the same conditions, generating the control necessary to observe and quantify the behavior of cell populations and single-cell arrays with biochemical assays[5]. In the future, we will use this system to create large arrays of single cells of various shapes for phenotypic studies and to create arrays of tissues with varying cellular organization.

Reflection on the UROP Experience

This UROP project was one of the main highlights of my sophomore year; one of my happiest memories from this semester is presenting my poster at the symposium. Aside from the accomplishment of completing my project, I learned a lot of new information about our lab's research and about the design process, and became much more experienced in the lab. A key example of this is that I learned how to use the microfluidic devices, and, of course, taught myself how to use them in parallel, skills I will continue to use in the lab. I became more experienced with many lab procedures, like fluorescent staining. I also learned about the design process while I was working on designing the system and I now have opinions about how I would choose to think about designing something in the future.

Acknowledgements

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