Flow Through Gels as a Tool to Generate 3D Concentration Profiles in Hydrogel-Filled Devices

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ABSTRACT

Laminar flow patterning is an iconic microfluidic technology used to locally deliver chemicals to specific regions on a two-dimensional surface with high spatial fidelity. Here we present a novel extension of this technology by flow patterning inside cured, three-dimensional (3D) hydrogels. We control the pressures at the inlets to shape the size and position of a region of high solute concentration in the main channel filled with a cured collagen 1 hydrogel. This method allows solutes to be delivered with 50 micron accuracy within the gel, as evidenced by control of concentration profiles of 40 kDa fluorescent dye.

INTRODUCTION

We present a novel technique for high-resolution control of two-dimensional (2D) and three-dimensional (3D) concentration profiles within a cured 3D hydrogel by through-gel laminar flow patterning. Specifically, we adapt laminar flow patterning, a technique typically used to manipulate concentration profiles over surfaces,[1] to the manipulation of concentration profiles inside cured hydrogels. To demonstrate control, we generate concentration profiles of 40 kDa and 1 kDa fluorescent dyes in collagen-filled microfluidic devices. Further, we extend this technique to control of 3D concentration profiles in a 3D printed device.

Our technique should be particularly useful for 3D cell culture. Even though microfluidic platforms to apply interstitial flow and concentration profile to 3D cell cultures already exist, [2][4] these only allow application of flow or gradients in the absence of the other. In living tissues, gradients in morphogen concentration are often coincident with interstitial flow[2] and our technique can facilitate research into the rich interplay between the two factors.

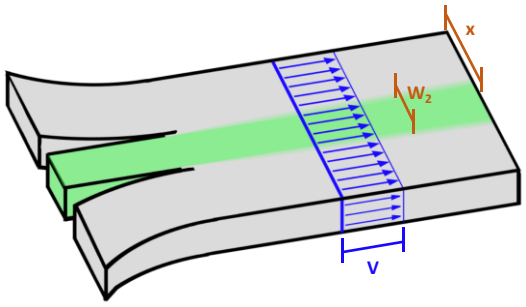

Figure 1: Schematic of PDMS device showing the hydrogel in gray, plug flow profile in blue, and the two geometric constraints, the width of the central streamline (W_2) and the lateral position of the stream (x). Pressures are modulated at the inlets (left) to guide the position of the concentration profile downstream. (green).

MATERIALS AND METHODS

Devices shown here were either fabricated of polydimethylsiloxane (PDMS) using soft lithography or 3D-printed in clear resin (Figure 1) with a Formlabs Form2 (Figure 3). Channel depth×width was 100 µm×1500 µm for the PDMS devices and 2 mm×2 mm for the 3D-printed devices. All devices were filled with a 4 mg/ml solution of Collagen I (Rat Tail, Corning) which was allowed to cure for 1 hour at 36°C. Flow through the gel was controlled via inlet reservoir pressures which were supplied by a Fluigent pump (MFCS-EX).

We demonstrate the technique by choosing several target concentration profiles which vary in both lateral position and profile width, and then configuring the inlet pressures to achieve these profiles in the device (Figure 2). We work within the physiologically relevant interstitial flow regime (10 µm/s)[3] with a 40 kDa dextran tracer dye chosen to match the diffusivity of the commonly used morphogen: vascular endothelial growth factor A.[7]

RESULTS AND DISCUSSION

We see excellent agreement between the target position and measured position of the stream (R^2=0.97; Figure 2, Left). It is worth noting that diffusion skews the profile towards the center of the channel when the profile is bounded on one side by a PDMS wall. This causes the slight deviation between target and measurement seen in the extremes.

To demonstrate control over the profile width, we compare the target profile width (calibrated for diffusion) and measured profile width and find a weaker correlation (R^2=0.78; Figure 2, Right), with the measured width consistently larger than the target. This deviation may be an artifact of slight errors in the lithography near where the inlets meet. The deviation appears to be consistent across devices cast from the same mold, which means that it will probably be possible to compensate for the deviation in future experiments.

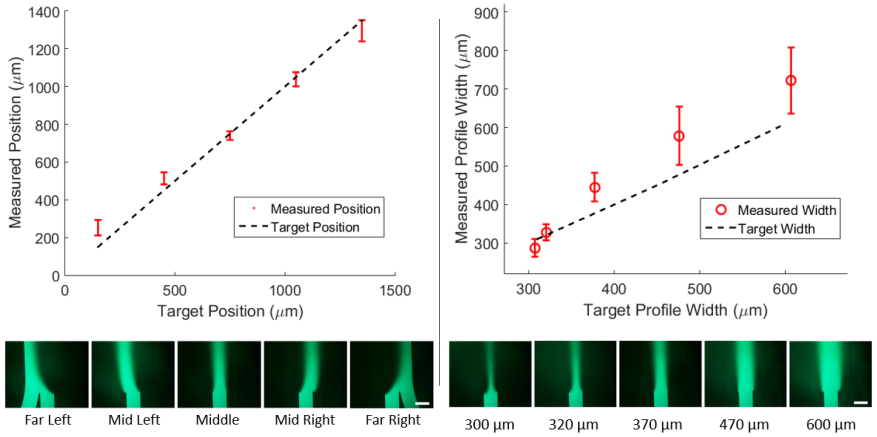

Figure 2: Positional and width control of the concentration profile within a collagen-filled microfluidic chip. Top left, measured center position of the concentration profile plotted against the target position. Top right, measured width of the concentration profile at 50% max intensity plotted against the target width at 50% max of the profile. N=3 devices for each data point shown, error bars are one standard deviation. Bottom, sample fluorescent images from the experiments. Inlets are at the bottom of the image, scale bars are 500 μm.

Finally, we present a 3D-printed device for control of the lateral and vertical position of the concentration profile (Figure 3). Similar to the 2D case, we applied different sets of inlet pressures and measure the resulting profile, this time from both the side and the bottom of the device. Results demonstrate that we are indeed manipulating the profile position in both the vertical and horizontal directions (Figure 3).

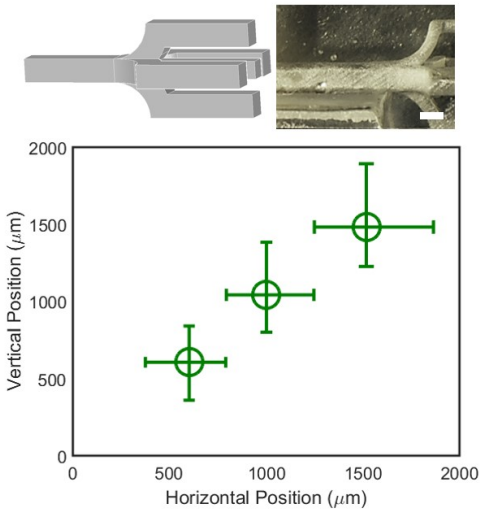

Figure 3: Top left, Rendering of the 3D junction. Top right, Picture of the 3D printed junction. Scale bar is 2 mm. Bottom, 2D position of the stream at a cross section for 3 different test conditions. Each point represents the vertical and horizontal position of the profile for a single inlet pressure configuration. Bounds of the graph are the bounds of the main channel. Error bars are one standard deviation of the respective fluorescent profile. Scale 500 μm.

CONCLUSION

Previously, laminar flow patterning has been used to locally deliver chemicals to flat surfaces in microfluidic devices. Here we have shown that 2D and 3D laminar flow patterning through a cured hydrogel is not only possible, but relatively simple to control. Through testing of our devices we demonstrate excellent spatial control of the generated concentration profile via manipulation of the inlet pressures and. Furthermore, we show that the technique can be used in a truly 3D capacity by 3D printing and testing a version of our device with five inlets for both horizontal and vertical control of the concentration profile.

AWKNOLEDGEMENTS

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