DATA-DRIVEN 3D INKJET PRINTING OF HYDROGEL MICROFLUIDICS
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ABSTRACT
Three-dimensional inkjet printing is a promising biofabrication tool for tissue engineering applications. In this work, we present a method of 3D printing a closed capillary in alginate and then plumbing it into a microfluidic setup in spite of its softness. The integrity and functionality of the microfluidic device was demonstrated by driving an aqueous solution through it at physiological flow rates. We believe this technique will enable researchers to rapidly prototype and produce vascularized heterogeneous tissue models for cell biology and drug discovery.

KEYWORDS: 3D printing, inkjet, biomaterial, hydrogel, tissue model

INTRODUCTION
Three-dimensional printing is used in industrial applications to rapidly prototype objects directly from CAD models, with individual droplets forming building blocks. It has also been proposed as a method for assembling artificial organs, as different channels of a color print-head could be used to print different cell and biomaterial types into heterogeneous architectures [1]. In this context, 3D printed hydrogel microfluidic systems represent an important step for two reasons. Firstly, researchers will need to optimize the precursors of repeating structures found in large organs, such as the nephrons in the kidney. Secondly, circulatory networks are a requirement for producing bulk tissues and would essentially consist of a plurality of interconnected branched 3D microfluidic networks [2].

Other soft-matter patterning techniques, such as extrusion, have been used to produce microvasculature in biomaterials. However, these typically require the removal of some casting material [3] before perfusion, or are unsuited for producing branched vasculature [4]. Furthermore, these patterning techniques lack the scale-up potential and multi-materials patterning capabilities of 3D inkjet printing.

Here, we demonstrate the drop-on-demand 3D printing of an alginate microfluidic channel. Next, to investigate how such a soft hydrogel structure might be feasibly handled and utilized for tissue models, the channel was plumbed into a microfluidic setup. Finally, physiologically relevant flows were passed through the setup to evaluate the integrity of the channel.

EXPERIMENTAL
The biomaterial ink or ‘bioink’ used in this work was a sodium alginate solution printed with a 50 µm diameter Microdrop Technologies inkjet head and a customized motorized stage setup (Figure 1A). The alginate was printed onto a gelatin substrate prepared with 10 mM CaCl2. Once alginate droplets arrived on the surface, Ca2+ ions diffused upwards from the gelatin; gelling the alginate and equilibrating the Ca2+ concentration (Figure 1B). Subsequent alginate layers were likewise gelled when printed onto previously gelled alginate layers. Fluorescent alginate was used to permit fluorescent widefield and confocal microscope observation under cell-culture conditions.

Each alginate channel was printed from a stack of .bmp files. Every .bmp file coded a layer of droplets with each black pixel representing an individual droplet (Figure 2A). An optimized printing algorithm was used to ensure pattern fidelity [5]. The droplets were usually printed at 50 µm intervals, with 10 µm sub-interval steps being used to close the overhanging regions of the channel [5]. Initially, closed 1.5 mm long test channels were printed and examined by confocal microscopy. Based on these results, 3.5 mm long 350 µm wide open-ended alginate channels were printed and integrated into the microfluidic setup.
RESULTS AND DISCUSSION

Each of the 1.5 mm long test channels produced had a nominal base diameter of 250 µm, corresponding roughly to 5 droplet diameters. Globally, the channels corresponded well to the model (Figure 2 B-D), and individual droplets were visible within the bulk in the confocal microscope images. Some minor tapering was observed near the ends of the channels, but the central diameter and channel height were both maintained.

Figure 2: A) Sample .bmp file used to print each layer. B) Rendered model of .bmp file stack showing internal void (grey). Vertical plane indicates axial slice. C) Comparison of planar slice near base of rendered vessel with confocal microscope image of printed vessel. D) Comparison of axial slice along center of rendered vessel with confocal microscope image of printed vessel

To aid the integration of the 3.5 mm long channels, the gelatin printing substrates were always prepared on the microfluidic connector assembly described in [6]. Inlet and outlet holes were punched in the gelatin, after which the inkjet was aligned and the alginate channel was printed between them (Figure 3A). The channels were enclosed by means of a purpose-cut PDMS gasket and a glass cover slip (Figure 3B). Warm gelatin was added to cut-outs in the PDMS gasket to form a seal at the center of the alginate channel once cooled. Simpler sealing protocols were tested, but these proved to be unreliable due to the large difference in stiffness of the materials in contact. For general reference, the Young’s moduli of the alginate, gelatin, and PDMS used were on the orders of <1 kPa, ~10 kPa, and ~1.5 MPa respectively, based on lab measurements and common literature values.

Figure 1: A) Printing of alginate vessel between inlet and outlet. B) Schematic of microfluidic plumbing with details of gelatin seal. C) Frames from fluorescent video microscope showing red fluorescent bead flowing through printed microfluidic channel.

To observe the flow in the channel, a suspension of red fluorescent microbeads was driven through the channel by means of a syringe in order to evaluate the integrity of the alginate channel under physiologically relevant flows. The motion of the beads recorded by fluorescent video microscope. By varying the syringe pressure, flow rates of several µm/sec to tens of mm/sec were obtained which represents the range of flows expected for small arteries and veins of the same diameter. The channel did not rupture under pressure (Figure 3C). Occasional failure at the boundaries between droplets was observed during accidental rough
handling of the printed channels during integration of the microfluidic systems. Accordingly, these devices were not used for measurements.

CONCLUSION

In this work, we succeeded in fabricating alginate microvessels using an adapted 3D printing strategy. This work demonstrates that hydrogel microfluidic vessels can be printed and perfused, validating 3D printing as an biofabrication technique for producing heterogeneous tissue models. Rather than printing different colored inks, an inkjet head might one day deposit different cells and biomaterials into a vascularized tissue precursor.

As this fabrication technique is data-driven, life-sciences researchers might build vascularized tissue models comprising heterogeneous biomaterial and cell patterns with the same flexibility that geometric models are currently prototyped for industrial applications.

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REFERENCES


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