A MICROFLUIDICS BASED 3D BIOPRINTER WITH ON-THE-FLY MULTIMATERIAL SWITCHING CAPABILITY

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ABSTRACT
This work details the development of a novel 3D (3-dimensional) printing system for use in the fabrication of complex biological tissue constructs. The printing system incorporates a microfluidic print head that uses coaxial flow focusing to generate, crosslink, and dispense a microfiber of sodium alginate, and a 3D positioning system to pattern the dispensed microfiber using fused filament fabrication methods, in a software configurable pattern. Valves are embedded in the print-head to enable switching between multiple material sources. Using this system, the composition of the dispensed fiber may be controlled on-the-fly during printing to create complex heterogeneous 3D structures.

KEYWORDS: 3D Bioprinting, Biofabrication, Coaxial Flow Focusing, Microfluidics, Tissue Engineering

INTRODUCTION
Recently there has been considerable interest in developing more advanced in vitro biological systems, particularly for drug development. Ideally, these biological systems would precisely mimic the in vivo human environment. Real human tissue is composed of a complex 3D arrangement of cells and structures, and recreating this arrangement is a logical first step towards fabricating realistic tissue structures. Much evidence has already been given that suggests that providing a 3D environment for the cells gives an improvement in the realism of the cellular response, as compared to a 2D cell arrangement [1,2].

One promising technology for creating complex 3D geometries is 3D printing. In our previous work, we described a new method of 3D printing biologically compatible hydrogel by using a coaxial flow focusing microfluidic print-head [3]. The print-head coaxially focused a stream of sodium alginate with calcium chloride (CaCl$_2$) causing the alginate to crosslink and then be dispensed as a gelled microfiber. Several others have used this technique to create microfibers [4,5], and have achieved promising results when embedding cells on or within the fibers, though the fibers were not patterned. The print-head was attached to a 3-axis positioning system and the fiber was deposited and stacked to achieve 3D macroscale structures. Because the CaCl$_2$ cross-linker is dispensed along with the alginate fiber, it will pool and disrupt the position of any already deposited fiber. We solved this problem by printing onto a porous membrane and applying vacuum to remove the excess CaCl$_2$, as shown schematically in Figure 1.

In this paper, we have built upon our technology by adding on-the-fly material switching functionality to the print-head. As seen in Figure 1, we incorporated pneumatic valves for each fluid channel in the print-head. Using these valves, we can both initiate and halt alginate gelation, and switch between multiple different alginate channels. The different alginate channels may contain suspensions consisting of different types of cells and any associated growth factors or other compounds.

PRINT-HEAD FABRICATION
Though our print-head is a microfluidic device, we did not use conventional photolithography based fabrication. As described in our previous work we used commercially available 3D printing (Objet 24) to create molds, then proceeded with standard polydimethylsiloxane (PDMS) casting techniques. There were two reasons we chose this method. First, in
agreement with others [6], we found that using cylindrical channels enabled more stable fiber transport and reduced clogging. Second, to achieve coaxial focusing, the alginate channel must have a smaller diameter than the CaCl$_2$ channels. Both of these features are difficult to achieve using conventional methods, but are trivial when using 3D printing. Because it is only possible to create semi-cylindrical channel structures on the printed molds, two complementary molds with male/female alignment features were created, and the two PDMS casts were bonded together to form fully cylindrical channels using a 1 minute exposure to clean air plasma at 700 mTorr.

Each integrated valve was composed of a thin PDMS membrane. When pneumatic pressure was applied, the membrane deflected and blocked the channel beneath it. The valve fabrication is shown in Figure 2. To create the thin PDMS membranes we used a 3D printed table-like structure and placed it on top of one of the complementary print-head molds. The cylindrical extrusions on the table-like piece created a 150 µm gap with the surface of the mold, in turn creating 150 µm membranes after PDMS was cast. At this membrane thickness, we found that the pressure required to fully activate the valve was often sufficiently high to damage the membrane (estimated at over 2 Bar). To reduce actuation pressure, we designed the channel region directly below the membrane to have a raised bowl shape, as shown in cross-section in Figure 3. This reduced the total deflection distance for the membrane and allowed the valve to be fully closed at 500 mBar of applied pressure, given a 70 mBar fluid channel pressure.

Figure 2: The print-head fabrication process. 1) Complementary molds (a, b) are designed in software, and 2) printed using 3D printing. 3) The table-like piece is placed on mold ‘a’ and PDMS is cast onto both molds and cured. 4) The PDMS pieces are plasma bonded together, and 5) the final assembled print-head with a manually cut tapered tip. The inset 1a) shows the table-like piece, and 1b) shows the mold for the raised bowl shaped feature.

Figure 3: A cross-section view of the valve membrane and raised bowl-shaped feature.

RESULTS AND DISCUSSION

3D structures were printed using fused filament fabrication methods employed by many commercial 3D printers. The continuously deposited alginate fiber was patterned in 2D layers and stacked to reproduce the desired 3D structures. Open source software (Slic3r) was used to generate a toolpath (g-code) from standard 3D CAD STL files, and a custom g-code interpreter translated the toolpath into movement commands specific to the positioning system. This method enabled 3D structures with arbitrary geometries to be realized, with the exception of overhanging features, since currently no sacrificial material is available. As a demonstration, the letters “UBC” were printed and can be seen in Figure 4. All printed samples were composed of 1 wt% alginate and gelled using a 125 mM CaCl$_2$ solution.
Because our print-head is able to switch the deposited material on-the-fly, continuously printed heterogeneous structures are possible. As a demonstration, a multi-colored cube and cylinder were printed, as seen in Figure 4. In these examples, two colored alginates were alternately deposited at regular intervals along the vertical axis. We are currently investigating inter-layer material switching, which would allow more complex structures such as vertically coaxial cylinders to be created. A blood vessel is a good example of such a structure. Despite being formed of a single stacked fiber, the structures are physically robust enough to manipulate with tweezers, and in the case of the solid cube, slice into thin sheets with a scalpel. In addition, the structures maintain their integrity for several months when submerged in water, but are easily dissolved using a calcium ion chelator such as Ethylenediaminetetraacetic acid.

Figure 4: a, b, c) CAD representation of the letters “UBC”, and a heterogeneous cube and cylinder, respectively. d, e, f) The 3D printed recreation of the CAD files, constructed out of alginate. The scale bars represent 5 mm.

CONCLUSION
We have designed and implemented a novel 3D printing system capable of creating software defined heterogeneous structures composed of the biocompatible hydrogel sodium alginate. The device was itself fabricated using molds that were 3D printed as this enabled the fabrication of microfluidic structures that are otherwise very difficult to realize. Lastly, we demonstrated software programmable on-the-fly material switching and patterning using the print-head to create heterogeneous hydrogel structures using CAD and fused filament fabrication techniques. Though the next step in this work is to investigate cell-laden hydrogel structures, these results represent a considerable step toward the general biofabrication of software configurable 3D tissue constructs with broad applications in biology, drug testing and discovery, and ultimately the fusion of living systems with other MEMS based devices.

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REFERENCES

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