3D Printing of Complex Scaffolds Using Freeform Reversible Embedding of Suspended Hydrogels (FRESH)

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Statement of Purpose: In the past decade, bioprinting has evolved to allow the creation of complex constructs for tissue engineering. However, bioprinting and layered deposition of soft hydrogels with elastic moduli less than 100 kPa to form a 3-D object still faces three key challenges, namely (i) keeping cells alive during printing, (ii) avoiding deformation in the printed material, and (iii) the removal of support materials used in maintaining print geometry during gelation. These challenges persist across commercial and open-source solutions for bioprinting. Our hypothesis is that bioprinting in a pre-existing, ubiquitous, pseudoplastic bath will provide support for fabrication of complex scaffolds with intricate, biomimetic geometries. To test this, we have developed a process termed Freeform Reversible Embedding of Suspended Hydrogels (FRESH) that leverages open source hardware and software to bioprint 3-D tissue engineering scaffolds using biocompatible materials and processes. Uniquely, the support bath is thermo-reversible at body temperature (37°C), so after printing the support can be easily removed without damaging the biopolymers or cells integrated into the construct.

Methods: The FRESH pseudoplastic support bath was produced by hydrating gelatin powder in calcium chloride solution and blending the resulting slurry to reduce particle size. Blended gelatin slurries were centrifuged until compaction and transferred into a Petri dish to serve as a support bath for the 3-D bioprinted materials. We performed proof-of-concept experiments using a print material consisting of a mixture of alginic acid and hyaluronic acid. A small percentage of the alginic acid was labeled with fluorescein using EDC and NHS to facilitate visualization with a fluorescent microscope. The alginic acid mixture was loaded into a MakerBot Replicator 3D Printer with a custom-designed syringe pump extruder for bioprinting. Digital models of scaffolds such as bifurcated tubes, arterial trees, and porous solids were generated using CAD software, MRI imaging data and/or Meshlab and then processed to G-Code using Skeinforge. ReplicatorG software was used to control the MakerBot and print the alginate. The 3D printed gels were "released" from the gelatin support bath by heating to 37°C and washing out the liquefied gelatin. Prints were imaged using a Nikon confocal microscope.

Results: Alginic acid extruded into the support bath crosslinked to an alginate gel, which remained immobilized during printing (Fig 1a). The printed alginate gel was released upon warming to 37°C, at which point the gelatin support material liquefied and was aspirated to reveal the alginate print (Fig 1b). The extruded alginate gelled as filaments with diameters in the range of 100 to 200 µm depending on printer settings (Fig 1c). These filaments were able to fuse together with neighboring filaments and layered to create the 3-D printed objects. More complex scaffolds were created using MRI data of the human right coronary artery tree (Fig 1d). The MRI data was converted to a model of cardiac arterial vasculature, processed into G-Code and then 3-D printed with alginate to form a biomimetic arterial scaffold. Confocal imaging confirmed that the complex, curved geometries were accurately replicated with hollow lumen diameters as small as 1 mm.

Conclusions: We have demonstrated that FRESH is capable of 3D printing complex structures from soft hydrogels. The pseudoplastic support bath allows for the bioprinting of a wide range of geometries based on parametric models, exceeding the capabilities of current 3D bioprinters. Ongoing work is focused on expanding the materials that can be printed to include collagen and fibrin and integrating cells into the scaffolds to engineer functional, 3-D printed tissues.

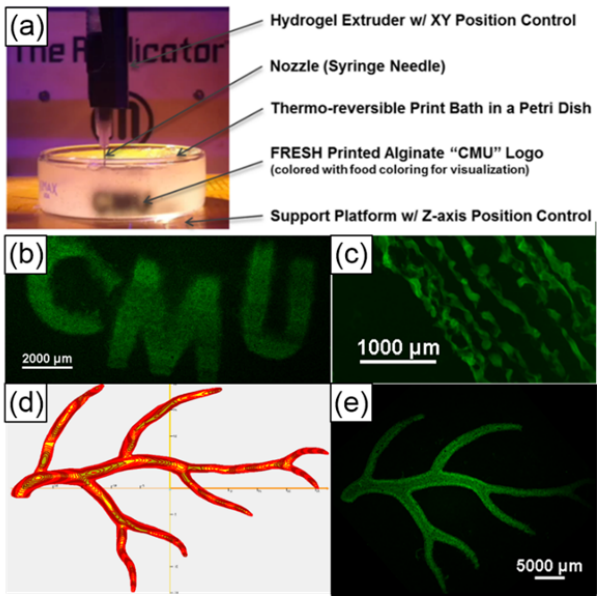

Figure 1. (a) FRESH printing consists of an XY syringe-based extruder that deposits hydrogels into a thermo-reversible print bath. The CMU logo (black for visual-ization) is printed at a height of ~8 mm. (b) Fluorescenct image of the printed CMU logo. (c) Filaments of deposited alginate gel. (d) A CAD model of the coronary vasculature obtained from MRI data. (e) The FRESH printed vasculature after thermal release from the print bath and imaged using a confocal microscope to show the hollow, manifold lumen with a diameter of ~1 mm.