3D printed Tissue Self Assembly via Embedded Printing of Newtonian Fluid Suspension Bioinks

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Introduction

In extrusion printing, it is crucial that the ink is shear-thinning, so that it can flow through a nozzle. It should also rapidly recover upon deposition in order to maintain its shape post-deposition. This shear-thinning and rapid recovery behavior limits significantly the materials that can be used in 3D printing, and in combination with the biocompatibility requirements, the available materials that can be used for 3D bioprinting are quite limited. Recently, embedded 3D printing has been developed based on granular and yield stress hydrogel support baths where materials can be added within the bath via extrusion, after which the support bath self-heals and supports the added material. This enables the 3D printing of materials that normally do not recover to a solid-state quickly enough after deposition, however the same prerequisites of shear thinning and shear recovery still apply. Newtonian fluids can readily flow through an orifice since they exhibit constant viscosity, and homogenous suspensions of particles with a volume fraction (φ) of $\varphi<0.1$ in a liquid, behave predominantly as Newtonian fluids. However if the φ of the particle suspension drastically increases after deposition, especially at $\varphi>0.64$, the suspension transitions into a solid, that can maintain its shape. Diffusion can be utilized as a method to reduce the liquid content of a suspension. Utilizing the synergy of these phenomena, we report the successful embedded 3D printing of Newtonian fluids and dilute solid suspensions such as microgels, cell spheroids, and cellular particle suspensions within yield stress fluid self-healing embedding baths. Tissue structures were 3D printed and extracted by diluting the support baths after 7 days of cell culture.

Materials and Methods

For the yield stress fluid embedding bath, 1.5% w/v Xanthan gum (G1253, Sigma-Aldrich, USA) powder was dissolved in mili-Q water, or a cell proliferation medium for the tissue fabrication experiments. For the suspension bioinks, 0.25% Alginate microspheres (IamFluidics B.V.) coated with PLL-FITC, polystyrene particles stained with coumarin-6, RFP-SMC spheroids and purified cardiomyocytes, were tested for the printing demonstrations and tissue fabrication. Blunt tip straight and 90° bent nozzles with ID ranging from 200μm to 600μm were used in the 3D printer (ROKIT INVIVO). Multicolor fluorescence microscopy imaging was performed in a custom built imaging setup, that allows real-time visualization of embedded injection on a moving stage. For the visualization, a 0.1% w/v Rhodamine in Mili-Q solution including polystyrene particles stained with coumarin-6, at a $\varphi=0.1$ was used to image the suspension ink.

Results

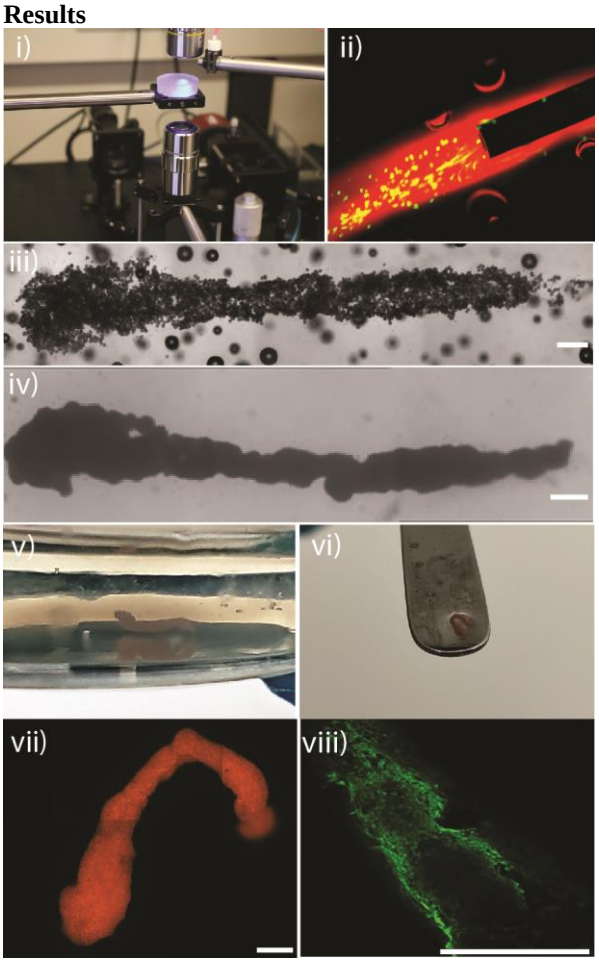

Figure 1: i) Custom imaging setup for real-time visualization of embedded injection. **ii)** Image frame of printing a suspension of polystyrene particles (green) in a rhodamine liquid phase (red) within a xanthan gum embedding bath. **iii)** 3D printed line of SMC spheroids in Xanthan Gum bath immediately after printing. **iv)** Compacted SMC tissue fiber at Day 4 of cell culture. **v)** Macroscopic image of tissue fiber at Day 7. **vi)** SMC fiber extracted with a spatula from the xanthan bath. **vii)** Confocal imaging of SMC fiber after extraction at Day 10. **viii)** Confocal imaging of SMC fiber with Actin staining showing cellular alignment and no spheroid pattern indicating successful fusion. Scale bar indicates 500μm in all images.

Summary

We demonstrate a method for 3D printing Newtonian liquid inks within a yield stress fluid embedding bath. In our work, we challenge the notion that bioinks should have shear-thinning and shear recovery properties. Instead we use dilute suspensions as inks, which is the most common state of matter during cell culture protocols. By utilizing the diffusive properties of liquids within hydrogel matrices, the dilute suspensions become *in-situ* jammed particle constructs. When cells are included, we observed dense tissue self-assembly that can be extracted after maturation.