



## Biofabrication of Three-Dimensional Tissue-Engineered Vascular Constructs

Changxue Xu\*

### Introduction

Organ transplantation has been successfully developed to save numerous patient lives in the last several decades. However, its limitations are becoming more and more conspicuous, such as immune rejection, and particularly organ donor shortage. For example, U.S. Department of Health & Human Services reports that around 22 people die each day due to organ donor shortage. Fortunately, organ printing offers a great potential for the fabrication of three-dimensional (3D) living tissues and organs based on precise layer-by-layer deposition using various tissue spheroids as building blocks. The fabricated tissues and organs are envisioned to be used for the replacement of damaged or injured human organs, providing a promising solution to the challenge of organ donor shortage. Vascularization has been widely recognized as a main technological barrier for building thick tissues and organs. Thus, the capacity to print 3D vascular constructs is not only a logical initial step but also a very important indicator of the overall feasibility of proposed organ printing technology. Our research interests focus on biofabrication of 3D vascular constructs using different advanced manufacturing technologies including inkjet printing, microextrusion, and stereolithography. In inkjet printing of 3D vascular constructs, we investigated the ligament pinch-off [1], cell-laden droplet formation process [2-4] and manufacturing process [5-7]. In ligament pinch-off study [1], four types of pinch-off were observed during inkjet printing of alginate solutions (viscoelastic biological material), and dimensionless numbers were employed to compare the relative magnitudes of different stresses including inertial, capillary, viscous and elastic stresses. It was found that the ligament exponential thinning in exit-pinch-off is quantified by the apparent relaxation time instead of the longest relaxation time due to the high frequency of pressure waves. Phase diagrams in terms of the dimensionless numbers were constructed to classify different regions for different types of pinch-off. In the cell-laden droplet formation study, we focus on the behaviors of bioinks with cell concentrations in the range of  $0-1 \times 10^7$  cells/ml during inkjet printing [2,3]. The properties of bioink with different cell concentrations were characterized, such as viscosity, surface tension, storage modulus and loss modulus. The Jacqueline fernandezn, the effects of cell concentration were systematically investigated in terms of the droplet size, velocity, and breakup time and satellite droplet formation. Furthermore, the droplet formation during inkjet printing of cell-laden bioink is compared with that during inkjet printing of

the polystyrene bead-laden suspension under the identical operating conditions to understand the different effects of soft living cells and hard particles on the droplet formation process.

### The main conclusions are

1. As the cell concentration of bioink increases, the droplet size and velocity decrease, the formation of the satellite droplet is suppressed, and the breakup time increases.
2. Compared to the hard bead-laden suspension, the bioink with the same cell concentration tends to have a less ejected fluid volume, lower droplet velocity decrease, and longer breakup time.

It is always a big challenge to print bioinks with a high cell concentration due to the large viscosity and elasticity. We proposed the electric field-assisted droplet formation method under piezoactuation-based inkjet printing to successfully print the bioink with a cell concentration of  $3 \times 10^7$  cells/ml [4]. The Taylor cone is intentionally suppressed to avoid undesirable satellite droplets. In the manufacturing process study, vertical printing and horizontal printing were proposed for fabrication of 3D vascular constructs, and the associated manufacturing challenges are analytically modeled [5-7]. It has been found that:

1. The maximum achievable height of overhang structure depends on the inclination angle of the overhang structure during vertical printing;
2. The cross-sectional deformation of the 3D vascular constructs is theoretically modeled based on Castigliano's second theorem, and the experimental results and model predictions are compared to show a good agreement;
3. A predictive compensation approach has been proposed to mitigate the cross-sectional deformation during horizontal printing to show a good result.
4. Alginate cellular tubes have also been successfully printed with a satisfactory post-printing cell viability of 87% immediately after printing and after 24-hour of incubation.

In Microextrusion of 3D vascular constructs, we proposed a microgel-assisted microextrusion method to fabricate 3D vascular-like constructs of interpenetrating network (IPN) hydrogels [8]. The microgel exhibits a characteristic yield stress behavior. As the nozzle moved at a specific speed inside the microgel, the shear stress overwhelmed the yield stress of the microgel, which resulted in the local fluidization of the microgel facilitating the bioink extrusion from a microextrusion nozzle. After the nozzle moved away, the microgel rapidly solidified to support the extruded filament in its wake. 3D vascular constructs were fabricated based on the layer-by-layer mechanism. It is noted that the fabricated 3D structures inside the microgel was able to maintain their shapes due to the microgel unique property of yield stress, although the ink was still a fluid, and uncross linked. Then, the printed 3D structures were subject to two-step gelation as whole units without interfaces between the adjacent layers, which significantly improved the mechanical properties of the fabricated 3D vascular constructs. In stereolithography of 3D vascular

\*Corresponding author: Changxue Xu, Department of Industrial, Manufacturing, and Systems Engineering, Texas Tech University, Lubbock, Texas, USA, Tel: 806.742.3543; E-mail: changxue.xu@ttu.edu

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Figure 1: 3D vascular constructs fabricated using different advanced manufacturing technologies: (a) Inkjet printing, (b) Microextrusion and (c) Stereolithography.

constructs, a 3D bioprinting system based on dynamic optical projection stereolithography was adopted to fabricate 3D vascular networks. The digital micromirror device (DMD) was utilized as a dynamic mask to create 2D patterns of the photocrosslinkable bioink, and 3D vascular networks were built in a layer-by-layer manner. The cure depth under different UV intensities was systematically studied and it was found that the cure depth was proportional to the natural logarithm of the ratio of the UV energy input and the critical energy input. The fabricated 3D vascular networks had three different types of channels: the large one with an outer diameter of 3.6 mm, the medium one with an outer diameter of 2.2 mm, and the small one with an outer diameter of 1.4 mm. Fibroblasts were encapsulated into the fabricated 3D vascular networks, and the post-printing cell viability was measured to be above 90%.

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**Author Affiliation**

[Top](#)

Department of Industrial, Manufacturing, and Systems Engineering, Texas Tech University, Lubbock, Texas, 79409, USA

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