JET-BASED 3D PRINTING OF BIOLOGICAL CONSTRUCTS

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Abstract

Organ printing is the layer-by-layer bottom-up fabrication of complex cellular organization of native tissues or organs by bioprinting multiple cell types and other biomaterials at designated positions. The rising success rate of transplants has resulted in a critical need for more tissues and organs. Approximately 95,000 people are on the waiting list for new organs in the U.S. alone, and some die every day waiting for transplants. Integrated with a better understanding of multicellular self-assembly, bioprinting-based organ printing provides a promising solution to the problem of organ donor shortage. While some major challenges in bioprinting are biological such as endothelialization, vascularization, and accelerated tissue maturation, there is a critical need to create scale-up technologies for the robotic fabrication of hollow three-dimensional (3D) vascular constructs for use as the first step toward organ printing. Both inkjet- and laser- based bioprinting technologies have been explored as enabling bioprinting technologies, and complex constructs such as 3D vascular and vascular-like constructs have been successfully fabricated.

Introduction

During the past decades, the demand for organ transplantation has rapidly increased all over the world, which has far exceeded the availability of organ donors, resulting in a severe organ shortage crisis. In the United States, for example, the number of patients on the waiting list in July, 2014 has risen to over 120,000 while the number of organ donors is only less than 5,000 [1]. The ultimate goal of tissue engineering and regenerative medicine is to design and fabricate functional human tissues and organs suitable for the regeneration, repair, and/or replacement of damaged or injured human organs. In recent years, organ printing, among different tissue engineering innovations, is a layer-by-layer additive fabrication approach for making three-dimensional (3D) tissue and organ constructs using cellular spheroids or bioinks as building blocks. It has been envisioned as a promising solution to the problem of organ donor shortage.

Vascularization is often identified as a main technological barrier for building 3D organs, so the capacity to print a 3D cellular tube is a logical initial step towards the success of organ printing [Mironov2003]. In particular, vascular networks deliver necessary nutrients and oxygen to the organ systems in the body and are critical to the rise and survival of large-scale multicellular organisms. Therefore, robotic fabrication of 3D tubular constructs is of great interest for the overall feasibility of the envisioned organ printing technology.

Generally, the fabrication techniques of 3D constructs can be classified into two main approaches [Ringeisen2013]: solvent casting and direct writing. Direct writing or additive manufacturing has been favored due to its versatility and capability of complex construct fabrication without the need for part-specific tooling or masks. Three approaches of direct writing have been implemented for the fabrication of 3D tubular constructs: inkjet printing

[Boland2007] [Nishiyama2009] [Xu2012], laser printing [Yan2013], and extrusion [Norotte2009] [Skardal2010]. While each direct-write method offers unique capabilities and advantages, jet/droplet-based techniques in a drop-on-demand (DOD) fashion can be more easily implemented to fabricate complex and heterogeneous parts with a resolution defined by the size of each droplet. As such, jet/droplet-based techniques have been favored in various biofabrication applications [Schiele2010] [Riggs2011] [Ringeisen2013], such as inkjet printing [Boland2007] [Nishiyama2009] [Xu2012] and laser printing [Yan2013], demonstrating their potential as enabling tools for organ printing.

The objective of this paper is to introduce laser and inkjet printing techniques and their implementation, which have a great application potential for 3D tubular construct fabrication. Sodium alginate (NaAlg) solutions, in particular, 8% (w/v) for laser printing and 1% (w/v) for inkjet printing, were used as bioinks which form a hydrogel during printing. Calcium chloride ($CaCl_2 \cdot 2H_2O$) solution (2% (w/v)) was used as the cross-linking solution while having a secondary function to provide a buoyant supporting force during the printing of overhangs and spanning features. The calcium chloride solution can be easily flushed away from delicate or intricate structures after printing, overcoming some limitations of using alternative solid support materials. For living cell-based 3D tubular constructs, NIH 3T3 mouse fibroblasts (ATCC, Rockville, MD) were used due to their great relevance to vascular constructs.

The rest of the paper is organized as follows. First, the mechanism, experimental setup, and results using laser printing are introduced in detail. Then, the mechanism, experimental setup, and results using inkjet printing are presented. Finally, the conclusions on the fabrication of 3D tubular constructs using laser and inkjet printing are drawn, and the future work is proposed.

Laser printing

Mechanism and experimental setup

Matrix-assisted pulsed-laser evaporation direct-write (MAPLE DW), a typical laser-induced forward transfer (LIFT) practice, is of particular interest in our study as the laser-assisted printing technique [Lin2009]. During a typical MAPLE DW process, a laser pulse is focused perpendicularly through the backside of a quartz support-based ribbon, which consists of an optically transparent quartz disc and a coating material to be transferred as shown in Fig. 1 [Yan2013]. Sometimes, the coating material may also include a layer of energy absorbing material(s) between the quartz and it. The energy of the incident pulse is then absorbed by the ribbon coating to cause extremely localized heating and sublimation of a small portion of the coating, resulting in a small vapor bubble. The generated bubble further expands and may eject part of the coating material downwards, forming a jet/droplet for deposition or patterning.

Figure 1 illustrates a typical laser-assisted printing experimental setup and its main fabrication steps. The laser printing setup contained a 193 nm, 12 ns (full-width half-maximum) ArF excimer laser (Coherent ExciStar, Santa Clara, CA) and an optical beam delivery system. Quartz disc (Edmund optics, Barrington, NJ) with an 85% transmittance for 193 nm wavelength laser pulses was used to make the ribbon, which had the coating material on the other side. The relative motion between the ribbon and receiving container was controlled using *XY* translational stages. The downward motion of the platform, on which the construct was printed, was precisely controlled using a Z axis stage.

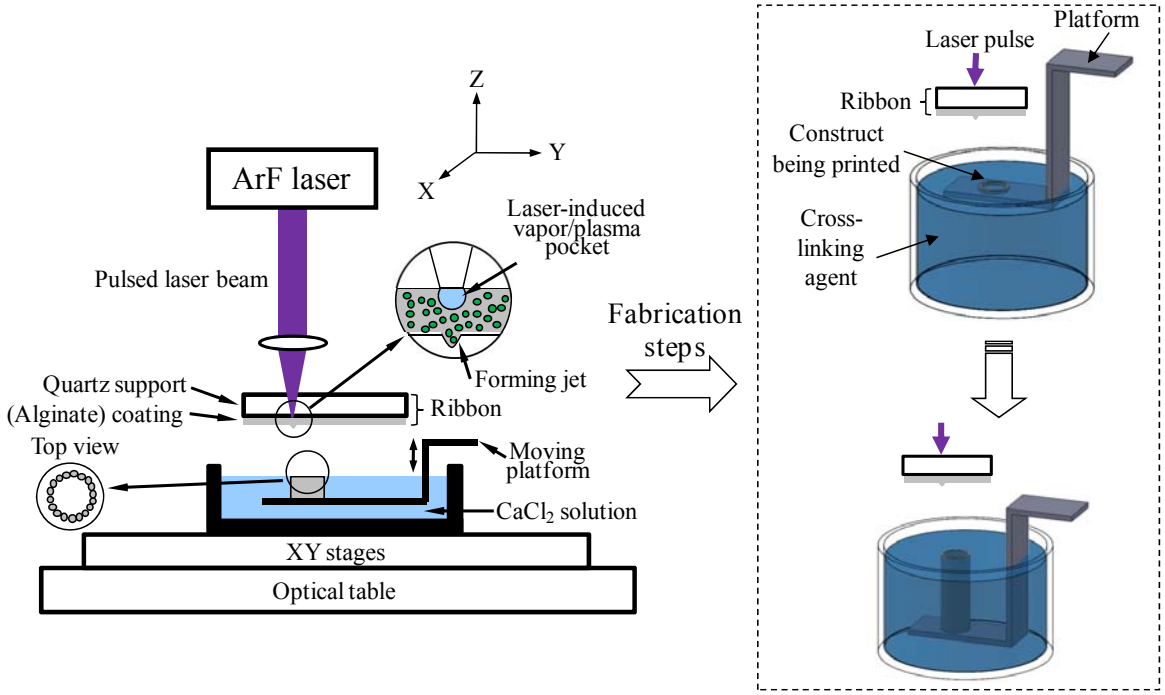

Fig. 1. Schematics of experimental setup of laser-assisted printing and its fabrication steps [Yan2013]

Experimental results

Figure 2(a) shows a representative alginate long tube printed using the proposed laser printing technique. The tube had a height of 6 mm and a diameter of around 3 mm, and it was fabricated by printing an 8% (w/v) sodium alginate solution into a calcium chloride solution under a laser fluence of 1,698 ± 45 mJ/cm^2. For further illustration, Fig. 2(b) and (c) show the top and side views of the tube [Yan2013].

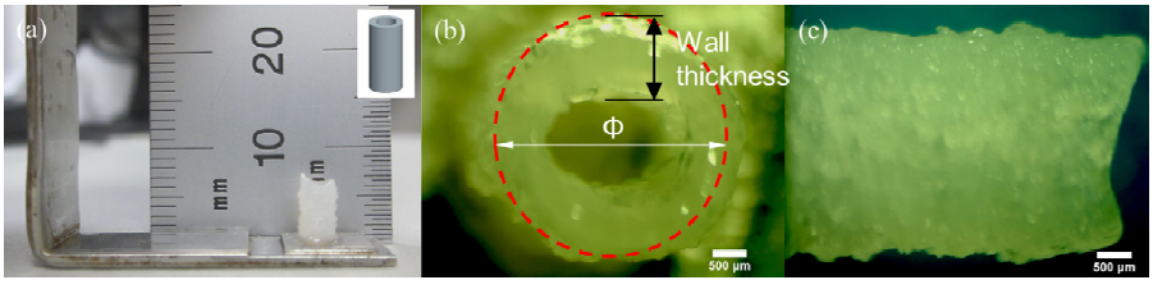

Fig. 2. (a) A representative tube fabricated by laser printing 8% (w/v) sodium alginate solution into calcium chloride solution, (b) top view of the tube, and (c) side view of the tube [Yan2013]

Inkjet printing

Mechanism and experimental setup

As shown in Fig. 3, a platform-assisted 3D inkjet printing system has been implemented for the fabrication of 3D tubular constructs. Sodium alginate solution with or without cells was

ejected to form droplets using a 120 μm nozzle dispenser (MicroFab MJ-ABL-01-120-6MX dispense head, driven by a sleeve piezoactuator) in a DOD mode. While depositing droplets, the nozzle trajectory for each layer was precisely controlled by a set of motorized *XY* stages. The construct was printed onto a substrate attached to a motorized *Z* stage, which was lowered into a calcium chloride solution for gelation by the layer thickness as designed. Based on codes developed in-house, the *XYZ* stages coordinated movements between the nozzle and the substrate to print 3D constructs. Overhang and/or spanning features can also be printed since the calcium chloride solution may provide a buoyant supporting force to them during printing if a fabrication process is well planned.

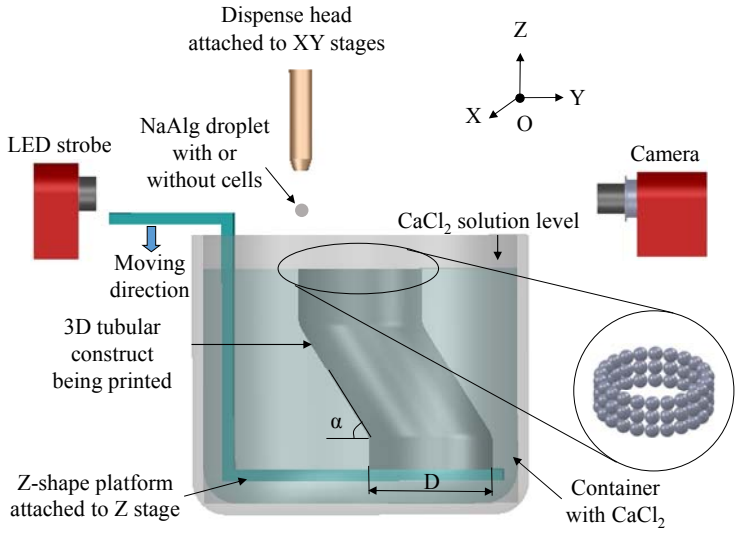

Fig. 3. Platform-assisted inkjet printing setup

Experimental results

For the proposed inkjet printing approach, tubular constructs may be fabricated through either horizontal or vertical printing configuration. For horizontal printing, the nozzle travels along the axial direction of tubes being printed. During horizontal printing, significant process-induced deformation may occur to constructs due to the elastic deformation of cross-section areas. By incorporating a predictive allowance for the deformation, horizontal tubular constructs can be successfully printed. As shown in Fig. 4 (a), nearly circular cross section has been achieved using horizontal printing.

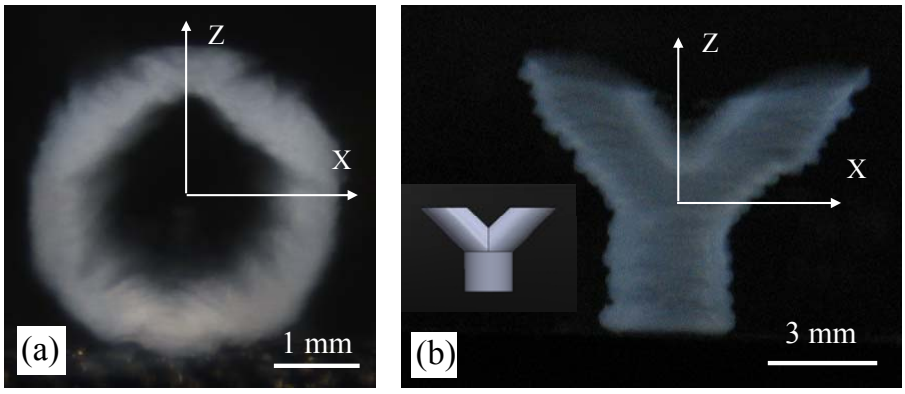

Fig. 4. (a) Cross section of the straight tube fabricated by horizontal printing with predictive compensation [Xu2013] and (b) branching tube fabricated by vertical printing

For vertical printing, the nozzle travels along the circumference of tubes being printed. During the vertical printing of tubular constructs with an overhang, two process failures might occur: structural instability due to the moment imbalance and structural failure due to the droplet impact-induced crash or buckling. For the former failure, the relationship between the maximum achievable overhand height and the inclination angle has also been derived [Xu2012]. As shown in Fig. 4 (b), vertical printing has been successfully applied to the fabrication of a bifurcated construct.

As shown in Fig. 5, inkjetting has also been implemented to print mouse fibroblast-based tubular constructs. The bioink used for printing consisted of 3×10^6 cells/mL (NIH 3T3 fibroblasts) inside 1% (w/v) sodium alginate-DMEM (Dulbecco's Modified Eagles Medium) solution. The printed cellular tube had a diameter of 3 mm and an inclination angle α of 63°, and satisfactory cell viabilities (above 85%) were observed immediately after printing as well as after 24 hours incubation.

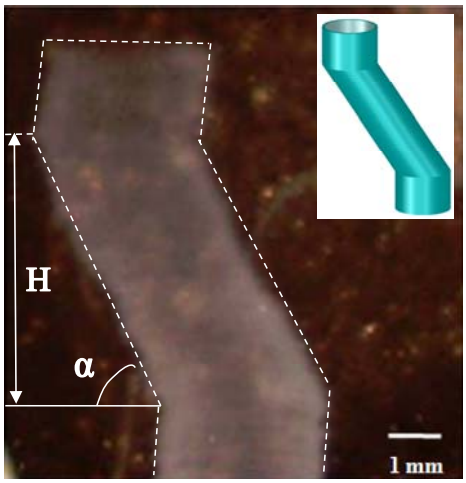

Fig. 5. Zigzag cellular tube fabricated from cell suspension with a 3×10^6 cells/mL cell concentration using vertical printing [Xu2012]

Conclusions

Vascular-like tubular constructs with and without cells can be successfully fabricated using on-demand laser and inkjet printing. It has been achieved by printing sodium alginate droplets with and without cells into a calcium chloride solution. The dual-purpose calcium chloride solution acts as a cross-linking agent and a liquid supporting material for some overhang and/or spanning features. The cell viabilities immediately after printing and after 24-hour incubation during inkjet printing are above 85% to show a satisfactory result.

Some future work may include: (1) cell damage evaluation during laser and inkjet printing, (2) theoretical modeling and/or computational simulation of process-induced deformation during horizontal printing, (3) fabrication of more complex constructs consisting of cellular heterogeneous multi-layered tubular constructs of varying diameters or more intricate geometries, and (4) tissue fusion of printed cellular constructs.

Acknowledgements

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