VERTICAL AND HORIZONTAL FABRICATION OF ALGINATE-BASED VASCULAR-LIKE CONSTRUCTS USING INKJETTING

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Abstract

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 bioink as building blocks. The capability to fabricate 3D cellular tubes is the first step as well as an important indicator of the overall feasibility of envisioned organ printing technology. In this study, vascular-like alginate tubes with a hemi-branching point, which mimic typical vascular constructs, are fabricated both vertically and horizontally using drop-on-demand inkjetting. In addition, manufacturing challenges associated with the vertical and horizontal printing configurations are briefly discussed. This study lays a foundation for the effective and efficient fabrication of viable 3D vascular constructs with complex anatomies (e.g. branching) as required in organ printing of vascular trees.

Introduction

The organ transplantation practice has been increasingly limited by the worldwide organ shortage reality and availability of organ donors, which calls for an alternative way to providing three-dimensional (3D) functional tissue/organ constructs for use. It has been a grand challenge in the scientific and engineering community that whether some 3D human tissue/organ constructs can be on-demand scaffold-free fabricated for implantation based on the computer-aided design (CAD) of needed tissues or organs [Mironov2008] [Schiele2010] [Riggs2011]. As such, organ printing, among different tissue engineering innovations, has emerged as a promising fabrication approach for making 3D tissue and organ constructs using cellular spheroids or bioink as building blocks.

Generally speaking, 3D complex structures/constructs can be fabricated in two ways: molding and additive manufacturing. In molding 3D structures such as cylinders are molded and fabricated with the aid of different molds made of Teflon [Khang2007], and silicon [Khang2007], to name a few. Unfortunately, the achievable shape and heterogeneity of parts limit broad applications of this technique. In additive manufacturing complex, heterogeneous parts are created without the need for part-specific tooling or masks, mostly through a layer-by-layer fashion. With increasing innovations, additive manufacturing has been providing a promising technological avenue for the fabrication of 3D human tissue/organ constructs.

The objective of this study is to investigate the feasibility in fabricating 3D vertical and horizontal vascular-like tubular constructs with a hemi-branching point using 3D inkjet printing, a versatile additive manufacturing technique with a great scale-up potential. The capability to printing 3D vascular-like constructs is not only the first logical step towards successful organ printing but also a critical indicator of the feasibility of the envisioned organ printing technology. Only are vascular constructs able to be on-demand fabricated, vascularization can be studied for better understanding of various organ printing-related clinical challenges. While vascular-like tubular constructs are fabricated in this study, the resulting knowledge in this preliminary study will facilitate the fabrication of 3D vascular trees.

Background

Most liquid-based additive manufacturing techniques used in 3D fabrication, which may be potentially applicable for biofabrication applications, can be classified into two types: droplet-based and filament-based. The former one can be implemented by using inkjet [Boland2007] [Nishiyama2009] [Xu2012] or laser [Schiele2010] [Riggs2011] to generate droplets as building blocks for deposition. The latter one is to use extruded filaments to make parts instead of using droplets, and this technology is represented by fused deposition modeling (FDM) [Zein2002], extrusion [Hamid2011], and micropen printing [Lewis2006]. Compared with filament-based techniques, droplet-based techniques have a high productivity and are easy to fabricate complex, heterogeneous parts with a resolution defined by the size of each droplet. As such, droplet-based techniques have been favored in many biofabrication applications [Boland2007] [Nishiyama2009] [Xu2012] [Schiele2010] [Riggs2011], and among them, inkjet-based technique is favored here for its scale-up potential, simple setup, and good process controllability [Herran2010] [Herran2012a] [Herran2012b] although it has a limited capacity in delivering highly viscous fluids, which is not of concern in this study.

Three-dimensional vascular or vascular-like tubular or branched constructs have been layer-by-layer fabricated using inkjetting [Boland2007] [Nishiyama2009] [Xu2012]. It should be noted that some vascular constructs were also fabricated via the assembly of preformed solid cellular rods [Norotte2009] [Skardal2010], which may not be easily extended to make 3D heterogeneous tissues or organs. Tissues and organs are composed of different kinds of cells, and the positions of cells and their supporting materials are delicately arranged within a 3D space, where they interact with each other and express their respective functions. Such 3D heterogeneous constructs, if expected, can be achieved using layer-by-layer additive manufacturing techniques such as inkjetting.

During inkjetting, 3D tubular constructs can be printed vertically or horizontally based on the relative configuration between the moving direction of dispense nozzle and the axis of tube being printed. If the nozzle moves along the circumferential direction of tube, the fabrication process is called vertical printing; if the nozzle moving direction is parallel to the tube longitudinal axes, the fabrication process is called horizontal printing. Most previous works on tube inkjetting were based on the vertical printing setup [Boland2007] [Nishiyama2009] [Xu2012], which can be easily implemented. However, it will be difficult in printing complex structures such as “Y” shaped structures if there is no sacrificial supporting structure during fabrication. There is a need to investigate the feasibility of horizontal printing and compare the performance of two printing setups, which is of interest in this study.

Inkjet Printing Setup and Experimental Conditions

Inkjet printing system

Tubular constructs with a hemi-branching point were printed using a platform-assisted inkjet 3D printing system [Xu2012], which had three key parts: motorized XY stages (Aerotech, Pittsburgh, PA) attached with a computer-controlled nozzle dispenser (MicroFab, Plano, TX), a motorized Z stage (Edmund optics, Barrington, NJ) attached with a Z-shape platform where constructs were printed, and a container containing the cross-linking solution. The bioink was ejected using a 120 µm MicroFab nozzle dispenser (MJ-ABL-01-120-6MX) in a drop-on-demand (DOD) mode. The DOD pulse was controlled using a MicroFab Jet Driver, and a pneumatic controller was used first to adjust the backpressure of the fluid reservoir to obtain an ideal meniscus for good droplet formation. The XY stages were precisely controlled to define the dispense head location for planar feature printing, and the Z stage was controlled moving down vertically to match the printing speed for each deposited layer. Ideally, once a layer is printed, the Z-stage attached platform should move down by a distance of the layer thickness for the gelation of each newly deposited layer.

Alginate, particularly, sodium alginate, has been used as a constituent of bioink in bioprinting [Nishiyama2009] [Xu2012]. While alginate is not an ideal material for living tissue construction, it is a good hydrogel material for proof-of-concept studies. In this study, sodium alginate (Sigma-Aldrich, St. Louis, MO) was also used to make 1% (w/v) sodium alginate bioink for vascular-like construct fabrication, and the cross-linking solution was 2% (w/v) calcium chloride (Sigma-Aldrich, St. Louis, MO).

Vertical printing setup

Under the vertical printing configuration, shown in Fig. 1(a), the dispense nozzle moves along the circumferential direction of tube during fabrication [Xu2012]. Tubular constructs were fabricated by layer-by-layer printing using the proposed platform-assisted 3D inkjet bioprinting system. During bioprinting, the alginate droplets were positioned precisely along the XY plane as the constituent elements to form a layer of annular pattern which was deposited on the top of a previous gelled alginate annular layer at a given tube height. Simultaneously, the Z-shape platform moved downwards to build the height of the printed 3D tube. Fig. 1(b) depicts a printing protocol applicable to both vertical and horizontal printing.

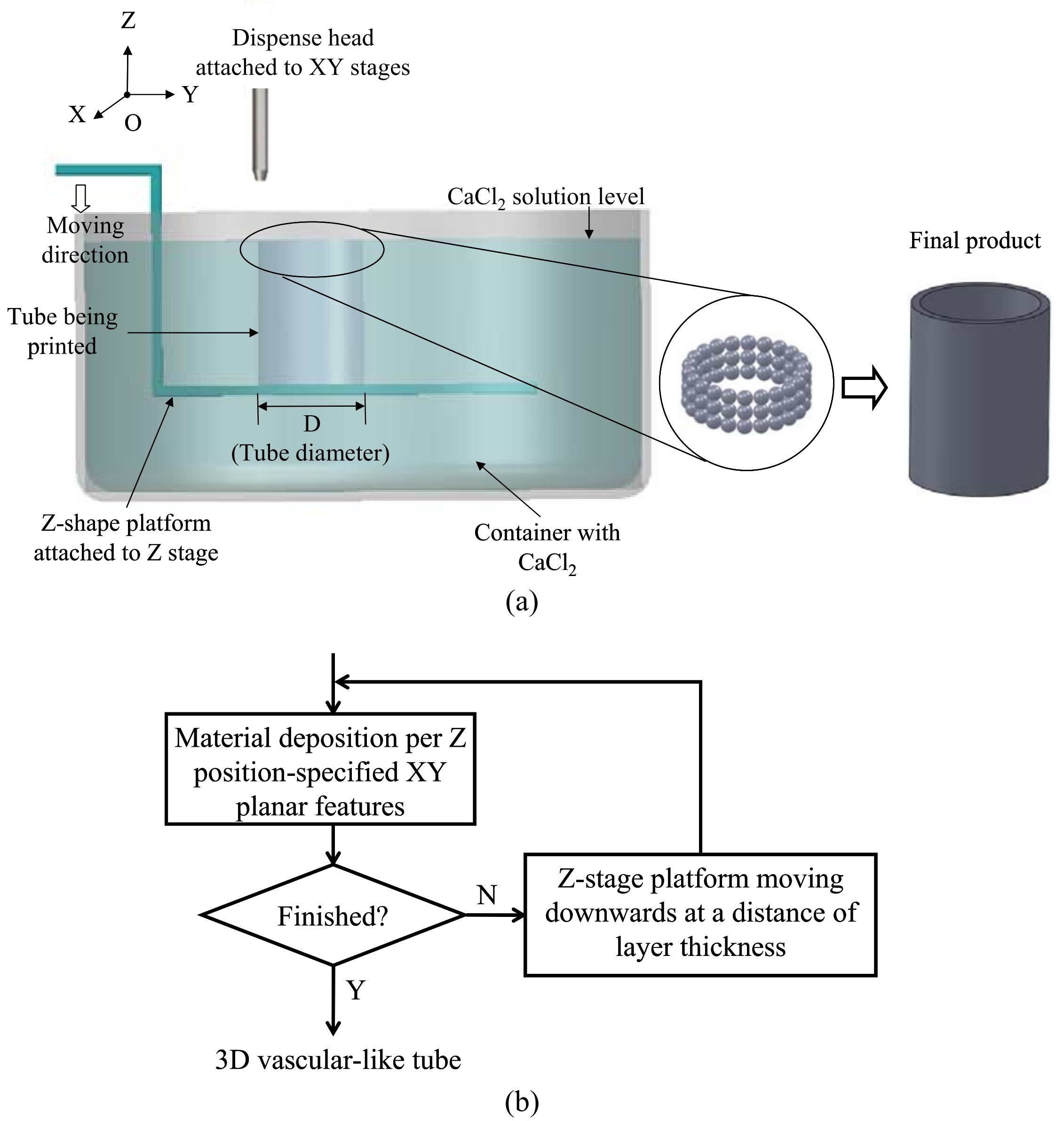

Fig. 1. (a) Vertical printing setup and (b) printing protocol

Horizontal printing setup

Under the vertical printing configuration, shown in Fig. 2, the dispense nozzle moves parallel to the tube longitudinal axes during the fabrication of each layer. As in vertical printing, the alginate droplets were positioned precisely along the XY plane as the constituent elements to form a layer of polygonal pattern which was deposited on the top of a previous gelled alginate layer at a given radial height of tube. Simultaneously, the Z-shape platform moved downwards to build the height of the printed 3D tube.

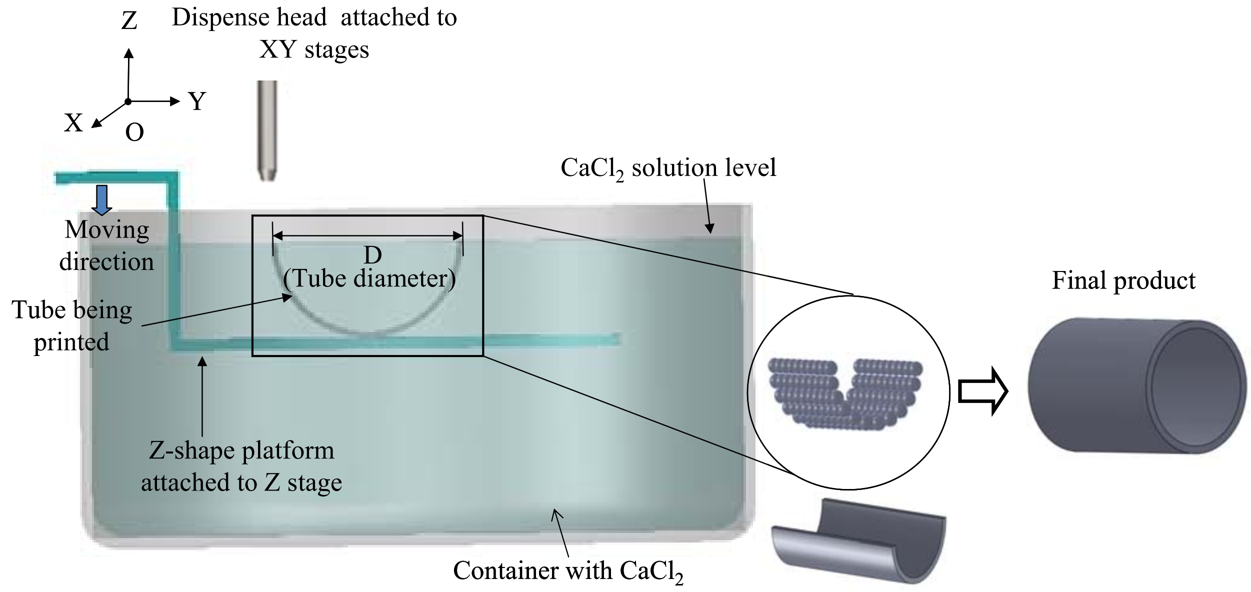

Fig. 2. Horizontal printing setup

Experimental conditions

During DOD inkjet printing in this study, the droplet formation was driven by the pressure of sleeve piezoactuator upon the external electrical excitation. Generally speaking, the dispense nozzle can be driven by different excitation waveform such as a bipolar waveform which consists of a succession of two square-wave pluses: either positive/negative or negative/positive [Herran2012a] [Herran2012b]. For the bipolar waveform, the second pulse of the wave is used to cancel some of residual acoustic oscillations that may remain in the dispense nozzle after droplet ejection. The bipolar excitation waveform used in the experiments is defined as follows [Herran2012a] [Herran2012b]: excitation voltages 60 V and -50 V, voltage rise or fall times 5 μs, dwell time 30 μs and echo time 20 μs. The printing resolution is limited by the droplet size which was on the order of 120 μm (orifice diameter) and is a function of liquid rheological properties and excitation waveform. Some other key experimental conditions are summarized in Table 1.

Table 1. Experimental conditions

Experimental parameters	Value
Printing frequency	100 Hz
Air gap between nozzle tip and $CaCl_2$ level	5 mm
Nozzle translational speed (within the XY plane)	150 mm/min
Platform speed (moving downwards at a constant speed in this study)	20 μm/s

Experimental Results and Discussion

Tubular constructs with a hemi-branching point were fabricated using layer-by-layer vertical and horizontal printing configurations, respectively. For a specific layer, its planar pattern was deposited on the top of the previously deposited layer, and all layers stacked and fused together, resulting in 3D constructs. In vertical printing the tubular structure was sliced into layers with a circular cross section while in horizontal printing it was sliced into layers with a polygonal cross section.

Vertical printing result and discussion

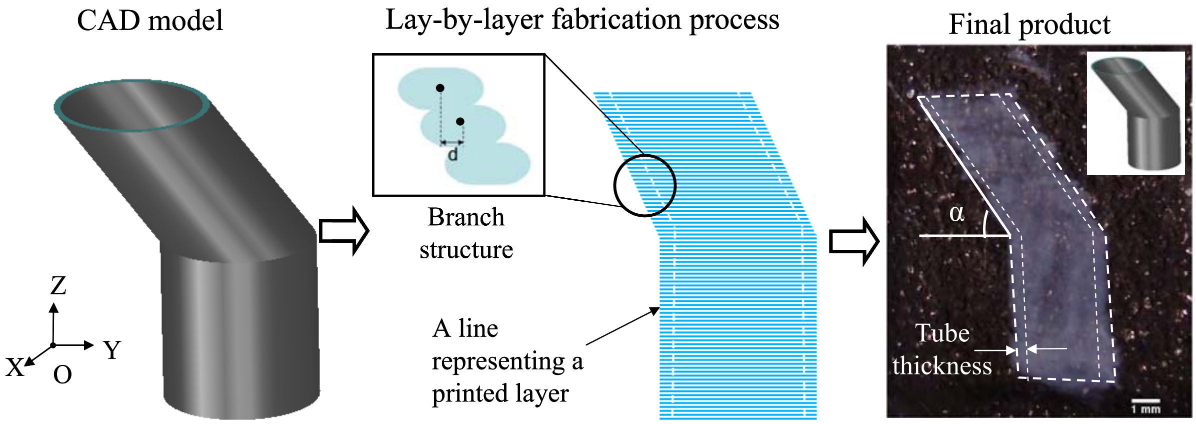

Fig. 3. Vertical printing of a tubular construct with a hemi-branching point

Fig. 3 illustrates the CAD model, the fabrication process, and the final product of a tubular construct (3 mm diameter) with a hemi-branching point made by vertical printing. The construct had two parts: the bottom straight part and the inclined branch part. During the printing of the bottom straight part, the horizontal displacement (d) of the successive layers was zero. During the printing of the branch part, the horizontal displacement of successive layers was 20 μm towards left, and the inclination angle (α) of the branch part shown was 53°. The inclination angle can be adjusted by choosing different combinations of the nozzle translational speed, the translational speed of the receiving platform, and/or the horizontal displacement of successive layers of the branch.

The branch structure was constructed by vertical printing and each layer stacked over each other to form a 3D structure. During the printing of branch structure, two process failures might occur: structure instability due to the moment imbalance and structural failure due to the droplet impact-induced crash or buckling. The former failure has been discussed and modeled by Xu *et al.* [Xu2012], which can be avoided by carefully selecting the branch structure height and the inclination angle. The latter failure occurs when the tube is too soft to resist any longitudinal deformation or buckling upon the impact force of droplet. This failure might be predicted based on the materials properties such as the Young's modulus and the geometry of tube wall for a given depositing droplet.

Horizontal printing result and discussion

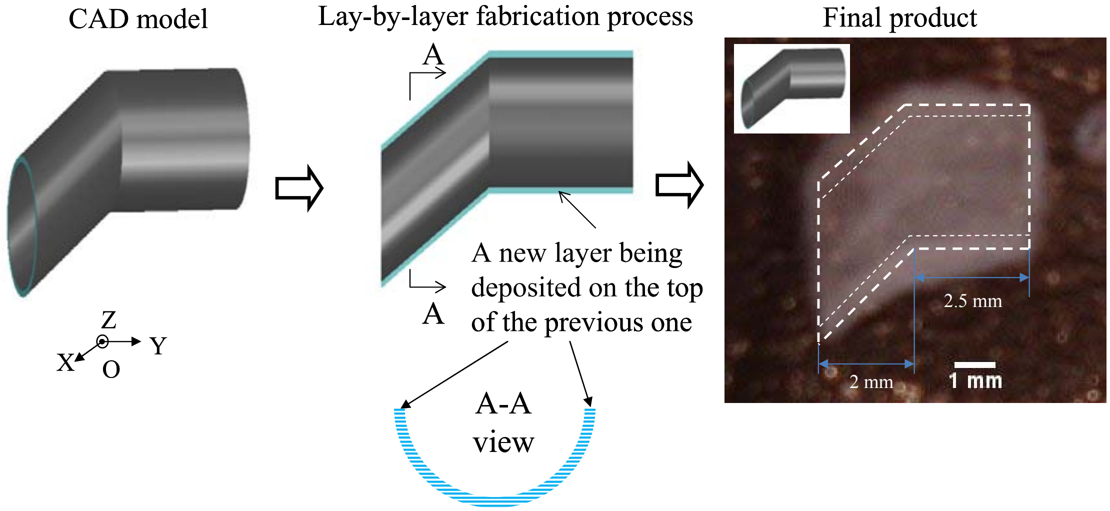

Fig. 4. Horizontal printing of a tubular construct with a hemi-branching point

Fig. 4 illustrates the CAD model, the fabrication process, and the final product of a tubular construct (3 mm diameter) with a hemi-branching point made by horizontal printing. As in vertical printing, the construct had the bottom straight and inclined branch parts with a 53° inclination angle. While the nozzle speed was 150 mm/min for feature printing, it should be noted that the nozzle speed was 600 mm/min at both ends of the tube. At this speed (600 mm/min), a gel line cannot be formed [Xu2012], so both tube ends were open as expected.

Different from vertical printing, the branch structure was printed horizontally, so the aforementioned structure instability and structural failure did not happen under this printing configuration. Instead, the main challenge in horizontal printing is how to layer-by-layer print a perfectly round tube, especially the top and bottom arc parts due to their small slopes. During the printing of the top and bottom arc parts, the horizontal displacement of successive layers might reach 420 µm for a 3 mm diameter tube, which is much larger than the feature size generated by a 120 µm dispense nozzle. As such, more layers instead of one were printed to achieve a necessary horizontal displacement, forming a continuous, solid arc. In order to maintain a constant tube thickness, the nozzle translational speed increased and the platform speed decreased accordingly to have a constant volume of material deposited per unit area.

Conclusions

Vascular-like tubular constructs with a hemi-branching point have been successfully scaffold-free fabricated by sequentially depositing sodium alginate solution into calcium chloride solution. It is found that the vertical and horizontal inkjet printing configurations are capable of making such tubular constructs with branches. Each printing configuration has its application limitations. It is difficult to make constructs with complicated branching structures such as the “Y” shape construct using vertical printing only. It is difficult to make a tube with a perfectly round cross-sectional shape using horizontal printing only. It is envisioned that truly 3D vascular constructs such as a vascular tree should be fabricated using the combination of vertical and horizontal printing.

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