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# Coaxial nozzle-assisted 3D bioprinting with built-in microchannels for nutrients delivery

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ABSTRACT

This study offers a novel 3D bioprinting method based on hollow calcium alginate filaments by using a coaxial nozzle, in which high strength cell-laden hydrogel 3D structures with built-in microchannels can be fabricated by controlling the crosslinking time to realize fusion of adjacent hollow filaments. A 3D bioprinting system with a Z-shape platform was used to realize layer-by-layer fabrication of cell-laden hydrogel structures. Curving, straight, stretched or fractured filaments can be formed by changes to the filament extrusion speed or the platform movement speed. To print a 3D structure, we first adjusted the concentration and flow rate of the sodium alginate and calcium chloride solution in the crosslinking process to get partially crosslinked filaments. Next, a motorized XY stages with the coaxial nozzle attached was used to control adjacent hollow filament deposition in the precise location for fusion. Then the Z stage attached with a Z-shape platform moved down sequentially to print layers of structure. And the printing process always kept the top two layers fusing and the below layers solidifying. Finally, the Z stage moved down to keep the printed structure immersed in the $CaCl_2$ solution for complete crosslinking. The mechanical properties of the resulting fused structures were investigated. High-strength structures can be formed using higher concentrations of sodium alginate solution with smaller distance between adjacent hollow filaments. In addition, cell viability of this method was investigated, and the findings show that the viability of L929 mouse fibroblasts in the hollow constructs was higher than that in alginate structures without built-in microchannels. Compared with other bioprinting methods, this study is an important technique to allow easy fabrication of lager-scale organs with built-in microchannels.



## 1. Introduction

Bioprinting (cell printing) is an exciting new technique that has been applied to the field of tissue engineering, in which layer-by-layer additive fabrication technology is used to directly deposit cells mixed together with biologically compatible hydrogel for fabrication of 3D tissues and organs. Many bioprinting methods have been tested as ways to fabricate cell-laden hydrogel 3D structures, including stereo-lithography [1,2], cell sheet lamination [3,4], inkjet printing [5–8], laser-assisted printing [9–12], and extrusion-based printing [13–17]. Compared with traditional scaffold-based tissue engineering approaches, bioprinting is an attractive alternative approach that allows fabrication of complicated tissues for major internal organs, handling and positioning multiple cell types, and the potential to integrate a vascular network in 3D tissue structures [18,19]. Because the cells require in vitro culturing prior to implantation, the fabricated 3D hydrogel structures must be adequately perfused to allow delivery of growth factors, oxygen, and other nutrients. Thus, the integration of a vascular network as occurs in thick tissues or organs is necessary and the most critical challenge in the successful application of bioprinting [20,21].

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Many approaches to the engineering of microchannel structures which can mimic a vascular network have been tested. A report from Huang et al. [22] that 3D zigzag cellular tubes were fabricated based on the fusion of sodium alginate droplets by using a Z-shape platform-assisted inkjet printing system. This method allowed scaffold-free fabrication of the tubular overhang structure and the post-printing 3T3 cell viability of printed cellular tubes was above 82%, but this method was limit in that it requires the precise deposition of sodium alginate droplets and requires long printing times. Another approach by Huang and colleagues [12] used a matrix-assisted pulsed-laser evaporation direct-write (MAPLE DW) to fabricate alginate tubes. Highly viscous materials such as 8% (w/v) alginate could be printed into well-defined long tubes and annular structures by this method, but cell survival was poor.

Recently, "modular" tissue engineering approaches [23] have been employed to fabricate macroscale tissue architectures by assembling shape and functional controlled microscale tissue building blocks, including vascular-like microchannels. Cyrille Norotte et al. [24] applied a fully biological, scaffold-free tissue engineering technology to fabricate small-diameter multi-layered tubular vascular grafts in a two-step process. First, cells were aggregated into discrete units, either multicellular spheroids or cylinders, and then they were printed layer-by-layer concomitantly with agarose rods, used as a molding template. Single- and double-layered vascular tubes were created by fusion of the discrete units. In an alternate strategy, Du et al. [25] presented an approach to rapidly build cell-laden microengineered hydrogel constructs embedded with vascular-like microchannels with circular lumen. Arrays of microgels with predefined internal microchannels were fabricated by photolithography, and then assembled into 3D tubular construct with multi-level interconnected lumens. However, both these methods require multi-step processes and expensive instruments.

Other vascular architecture fabrication methods based on sacrificial technology have provided a promising alternative [26–30]. Biocompatible hydrogel gels among various sacrificial materials are often used to create a temporary template. Seung-Schik Yoo et al. [26] reported a method to print hydrogel scaffolds containing fluidic channels using gelatin printed between collagen layers and then liquefied and drained at 37 °C to form a hollow channel within the collagen scaffold. Joshua Hammer et al. [27] presented a process to fabricate hydrogels with microchannel-like porosity in which stimuli-responsive calcium alginate microfilaments served as sacrificial materials for fluidic channels and were encapsulated within photocrosslinkable hydrogels composed of methacrylated gelatin (Gel-MA). Wang et al. [28] described a method using a crosslinked sodium alginate as a biocompatible sacrificial template to fabricate interconnected 3D microfluidic vascular networks in hydrogels. The sacrificial templates are rapidly replicated in PDMS microfluidic chips via $Ca^{2+}$-crosslinking, fully encapsulated in hydrogels, and then the interconnected 3D microfluidic channels were generated by dissolving the template with EDTA solution. In addition to hydrogel gels, other water-soluble materials can be used as sacrificial materials. For example, Jordan S. Miller et al. [29] presented a method based on carbohydrate glass as a sacrificial template. Willie Wu et al. [30] developed a method using wax-based ink as a sacrificial template which was liquefied when the temperature is raised to 80 °C. However, almost all of these methods are limited by complicated processes and require removal the sacrificial materials under conditions that do not permit cell viability.

Fluidic channels can also be formed in hydrogels by depositing the cell-laden alginate solution around a metal rod [31] or a Pt wire electrode [32] followed by removal of the templates to create hollow lumen in the vascular structure, but this method is able only to generate a single straight lumen with uniform size and morphology.

An electrospinning technique in which two immiscible liquids were electrospinned through a coaxial, two-capillary spinneret to fabricate several kinds of hollow nanofilaments has been successfully adopted in tissue engineering [33–35]. Inspired by the concept, some researchers used a coaxial nozzle which plays a key part in electrospinning equipment to fabricate micro-fluidic channels. Ibrahim T Ozbolat and workers [36] have explored the characteristics of the channels fabricated with a coaxial nozzle. They designed a pressure-assisted freeform fabrication platform combined with a coaxial nozzle dispenser unit to print hollow filaments based on the gelation process of sodium alginate and calcium chloride solution. Then they investigated the dehydration, swelling, degradation, perfusional, permeable, and mechanical properties of the printed microfluidic channels [37]. To enhance mechanical properties, they presented a fabrication process in which conduits were reinforced with carbon nanotubes (CNTs) [38]. They demonstrated that the bioprinting process could induce quantifiable cell death and that cells were able to recover and undergo differentiation with high-level cartilage-associated gene expression [39]. In another report, they designed a multi-arm bioprinter with two nozzles mounted on independent arms to concurrently print a filament structure and deposit cell spheroids between the filaments to create a hybrid structure to support the cell spheroids in three dimensions [40]. Their work presented a very useful method to fabricate fluidic microchannels as an alternative to biomimetically fabricated bifurcated vessels. However, in this method, hollow calcium alginate filaments are only used as microchannels and must be embedded into the multi-layer hydrogel. Many reports focus on bioprinting methods in which alginate microparticles or microfilaments are used as building blocks since they can fuse together to form tissue constructs [16,17,22,41], but the use of hollow alginate filaments in the fabrication of three-dimensional (3D) bioprinting structures has not been reported.

Here, a new bioprinting method based on hollow alginate filaments by using a coaxial nozzle is presented. High strength cell-laden hydrogel 3D structures with built-in microchannels were fabricated with fusion of adjacent hollow filaments by controlling the crosslinking time sequence. The hollow alginate filaments shown in this work serve as scaffold to support the mechanical integrity of the cellular environment in 3D tissue constructs, and can also act as built-in microchannels to deliver nutrients for cell growth. Compared with other bioprinting methods, this method allow concurrent printing of scaffold and microchannels.

## 2. Materials and methods

### 2.1. Materials

In this study, sodium alginate (Na-Alg) solution was prepared by dissolving Na-Alg (Sigma–Aldrich, Shanghai, China) into deionized water and placed in a magnetic stirrer for 24 h at 120 rpm at room temperature to make the final Na-Alg solution with a concentration of 2–5% (w/v). Similarly, calcium chloride ($CaCl_2$) solution was prepared by dissolving $CaCl_2$ (Sigma–Aldrich, Shanghai, China) into deionized water to make the final $CaCl_2$ solution with a concentration of 2–5% (w/v). Red and purple dye was added to the $CaCl_2$ solution to distinguish the core section from the sheath section of hollow filaments and was also injected to the fabricated microchannel for perfusion. Green fluorescence protein (EGFP , MW 27KD) (Miaoling Biological Technology Co., Ltd., WuHan, China) was perfused into the printed hydrogel structures as a model biomacromolecule to perform a permeability test.