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Design of a 3D printer head for additive manufacturing of sugar glass for tissue engineering applications

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ABSTRACT

Additive manufacturing is now considered as a new paradigm that is foreseen to improve progress in many fields. The field of tissue engineering has been facing the need for tissue vascularization when producing thick tissues. The use of sugar glass as a fugitive ink to produce vascular networks through rapid casting may offer the key to vascularization of thick tissues produced by tissue engineering. Here, a 3D printer head capable of producing complex structures out of sugar glass is presented. This printer head uses a motorized heated syringe fitted with a custom made nozzle. The printer head was adapted to be mounted on a commercially available 3D printer. A mathematical model was derived to predict the diameter of the filaments based on the printer head feed rate and extrusion rate. Using a 1 mm diameter nozzle, the printer accurately produced filaments ranging from 0.3 mm to 3.2 mm in diameter. One of the main advantages of this manufacturing method is the self-supporting behaviour of sugar glass that allows the production of long, horizontal, curved, as well as overhanging filaments needed to produce complex vascular networks. Finally, to establish a proof of concept, polydimethylsiloxane was used as the gel matrix during the rapid casting to produce various "vascularized" constructs that were successfully perfused, which suggests that this new fabrication method can be used in a number of tissue engineering applications, including the vascularization of thick tissues.

1. Introduction

The field of regenerative medicine, including tissue engineering and cell therapy, is directed towards the use or manipulation of living cells or tissues to treat disease. Traditionally, two-dimensional (2D) cell culture systems are used to produce and study cells with regenerative potential *in vitro*. However, these systems do not accurately replicate the complexity of native three-dimensional (3D) tissues. This complexity includes mechanical and chemical stimuli that affect the way cells function and behave. Thus, the ability to successfully culture cells in a 3D environment is critical to the advancement of regenerative medicine. This entails the adequate transport of nutrients, secretory and waste products, and signalling molecules within the 3D system. One major challenge in 3D cell culture and regenerative medical devices is achieving homogeneous oxygenation throughout the system. For example, one cell therapy approach to treating type 1 diabetes is to transplant insulin-secreting pancreatic beta cells encapsulated in a hydrogel matrix. Pancreatic beta cells have a high oxygen demand, which is essential for proper beta cell differentiation [1], development and function [2]. A lack of adequate oxygenation post-transplantation leads to impaired function (*e.g.* poor insulin secretion) and cell death, ultimately rendering the transplant ineffective [3]. This phenomenon is observed with other therapeutic approaches, such as hepatocyte transplantation to treat liver disease [4], as well as cardiac [5] and bone [6] tissue engineering. Consequently, various strategies have been proposed to vascularize engineered tissues in order to improve *in vivo* oxygen distribution and graft performance [7].

A popular approach is to engineer drug-eluting tissue constructs that promote *in vivo* vascularization. For example, scaffolds may be loaded with vascular endothelial growth factor, a protein stimulating the formation of new blood vessels [8]. Another approach is tissue bioprinting, whereby the cells are printed directly into desired locations of the printed construct. In this approach, a "bioink" composed of biomaterials and cells is deposited in a layer-by-layer fashion to produce a target structure [9]. However, bioprinting can be lengthy and cells may experience stress

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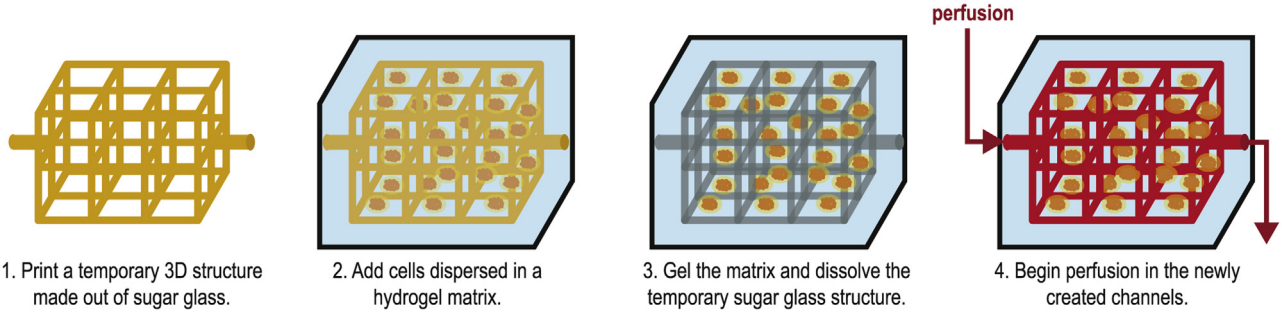

Fig. 1. Steps for fabricating a vascularized engineered tissue construct using a rapid casting method with sugar glass as a temporary lattice structure.

in the printing reservoir, during and after printing. The reliability of this approach and the durability of the tissue constructs obtained using bioprinting have not yet been demonstrated. While tremendous progress has been made in designing hydrogel-cell inks, incorporating vascular structures is proving more challenging. The bioprinting approach to vascularization often relies on the use of self-assembling components, such as vascular tissue spheroids [10]. These spheroids are printed, and over time fuse to produce tubular structures that may be perfused [10]. The drawback to both the drug-eluting and bioprinting approaches is that by the time perfusable vascular networks have been established, the graft has already been adversely affected by inadequate oxygenation.

One path envisioned to cope with this problem is to use additive manufacturing as a biofabrication tool. Additive manufacturing (AM) has been employed extensively to produce microfluidic devices and is foreseen to become an important tool for the development of many applications [11,12]. Many AM technologies have advantageously been used to make sacrificial templates to create channels within materials (*e.g.* PDMS, epoxy, pluronic gel, PEG hydrogel, and alginate) [13,14]. In addition, biofabrication has emerged as an interdisciplinary field combining cell and developmental biology, mechanical engineering as well as materials science and is expected to be "the dominant paradigm for 21st century manufacturing" [15]. The application of AM to biofabrication could indeed provide a platform to engineer tailored perfusable tissue constructs in the coming years. One way to obtain these constructs is through the rapid casting of fugitive inks, which builds on the work of Lewis et al. [16–20]. First, a vascular template is 3D printed using a fugitive ink that may be removed during a later step (Fig. 1). A matrix material containing the cells of interest is cast around the template. The template is then removed from the construct, leaving hollow channels mimicking blood vessels. This method requires a relatively short time to fabricate perfusable vascular networks, and therefore reduces the risk of graft damage due to hypoxia. Some proposed 3D printing fugitive inks include hydrogels such as agarose [21] and Pluronic F-127 [22]. However, many of these hydrogels are difficult to remove from the tissue construct, or may be cytotoxic [23]. An alternative fugitive ink is sugar glass, which is biocompatible and may be printed to form solid, brittle structures that can be easily removed by dissolution in an aqueous medium.

Although sugar glass printing and conventional extrusion 3D printing, such as fused deposition modeling (FDM), share come common features, several notable differences can significantly impact the printing strategy. During FDM, a solid polymer material is usually melted within the printer head and then extruded. In contrast, the sugar glass mixture must be prepared as a liquid prior to loading in the printer. The water content of the sugar glass mixture used as a fugitive ink and the temperature at multiple positions within the printer head must be controlled in order to achieve the desired viscosity, mechanical properties, rapid solidification, and consistent printing results. In addition to changing the water content, the residence time of the sugar mixture at a given temperature will affect its degree of polymerization [24]. Thus, the rheological properties of the sugar glass during printing and its mechanical properties after printing depend on the preparation procedure and the residence time in the printer head. Furthermore, sugar glass requires special storage considerations, as the material is highly hygroscopic.

Miller et al. have demonstrated a method of 3D printing sugar glass to form artificial vascular networks that may be perfused *in vitro* [25] and *in vivo* [26]. However, post-processing is required to remove extraneous filaments and printing has been limited so far to two-dimensional networks [25]. In contrast, native vasculature exhibits complex geometries, including curving and branching in three dimensions, which is necessary for proper nutrient and oxygen distribution in thick tissue constructs. With Miller's approach, sugar glass filaments ranging from 0.15 to 1.2 mm in diameter have been generated [25]. Artificial blood vessels smaller than 6 mm in diameter are prone to thrombotic occlusion *in vivo* [27], therefore it is necessary to be able to print larger filaments to preserve vascular patency.

This work describes the design of a 3D printer head intended specifically for the production of sugar glass structures. The aims of the design are to confer the ability to print complex 3D geometrical structures, and to increase the range of printable filament diameters. The results of this work build on previous sugar printing technologies by offering a robust method to print more complex temporary lattices for tissue engineering applications.

2. Material and methods

2.1. Design of the printer head

An existing open source 3D printer was improved and adapted to print sugar glass. This design decision allowed us to focus our engineering efforts on the customization of the printer head rather than on the X, Y, Z positioning system. The Airwolf3D XL (AW3D XL) was selected as the printer to be adapted since its architecture is simple and easy to adapt. This device's printing volume is $300 \times 200 \times 178$ mm, which is suitable for our applications.

Two major aspects needed special consideration during the design process of the new printer head. The first one was to find an appropriate way to stock the bulk sugar glass material prior to its extrusion. In FDM, the material is initially drawn into a constant diameter filament (typically 3 mm) and wrapped into coils that are then sold to the consumer. In sugar glass printing, the bulk material used is a mixture of glucose, sucrose and water heated to the appropriate temperature at a suitable rate. Since the raw material is in a liquid state, it cannot be stored in coils like the plastic filaments. Instead, the sugar glass mixture must be prepared immediately prior to every printing session. The liquid syrup