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 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, 17, 18, 19, 20]. First, a vascular template is 3D printed using a fugitive ink that may be removed during a later step (Figure 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 x 200 x 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 was therefore stored in a heated glass syringe, as proposed and described by Miller *et al.* [25], and a holding system was so designed that it could be easily integrated with the AW3D XL printer. The second aspect that was addressed during the design process was to find an effective extrusion technique to force the syrup out of the syringe. No commercial alternative suited all the requirements; therefore, a custom system was developed. In the design proposed in this paper, the syrup is pushed out of the printer head by moving the syringe's plunger using a motorized system. In the original approach described by Miller *et al.* [25], air pressure was used to push the syrup out of the syringe. Using precise and controlled plunger displacement resulted in a more accurate extrusion process but increased the technical challenges associated with properly filling and purging the syringe during setup. In our hands, the precision and design freedom obtained using a motorized plunger to produce temporary sugar glass structures far outweighed the burden associated with the additional tasks needed during the setup.

The AW3D XL printer moves the printer head in the X and Z axis, and the bed is motorized along the Y axis. The original printer head was completely removed and a new device, shown in its final form in Figure 2, was designed to ensure seamless integration with the AW3D XL. The main component of the new printer head is a 20 mL borosilicate glass syringe (Poulten & Graf GmbH, Air-Tite Products) which serves as the syrup reservoir. In order to maintain the syrup in a liquid state, precise and constant heating of the syringe was needed. Therefore, the support piece holding the syringe must withstand high temperatures (110°C). Furthermore, to facilitate manual manipulation, the outer surface should not exceed 60°C. In order to achieve these requirements, the syringe holder was designed based on a simple thermal model and then 3D printed out of ABS plastic. Air voids were included in the design of the syringe holder to ensure lower thermal conductivity and a lower surface temperature. Once printed, this component was fixed to the AW3D XL, enabling movements of the printer head in the X and Z axis.

The next challenge was to design a motorized translation stage that would allow precise movement of the plunger. Once designed, this stage was installed between the plunger and the holder as shown in Figure 2. The system uses a lead gear and worm gear (A1P6MYK08R030 and A1Y5MYK08RB, SDP-SI) coupled with an ultra-fine pitch screw (1/4-80 screw, Thorlabs Inc.) which allowed a reduction factor of 30:1 between the output of the motor and the screw. To ensure proper alignment with the syringe, the translation stage was positioned on three rectified rods used as linear guides, as shown in Figure 2. During extrusion, the translation stage pushed the syrup through a custom-made nozzle screwed to the end of the syringe. The extrusion stepper motor was used in quarter step excitation (800 steps/revolution) with a commercially available stepper motor driver (SainSmart A4988 Stepper Motor Driver). The reduction factor of the worm gear assembly (30:1), coupled with the ultrafine pitch screw, resulted in 1/24000 turn of the screw for each motor step. Since the internal diameter of the syringe was 19.3 mm and the diameter of the custom-made extrusion nozzle was 1 mm, a resolution of 0.0049 mm of material extrusion per motor step was obtained. For example, in this design, when the motor was run at 60 RPM (800 steps/sec), the extrusion rate was 3.92 mm/s. An advantage of such a design, compared to a direct drive design, is that the motor can be used at a much higher angular

velocity, therefore reducing the reflected load inertia (*i.e.* equivalent load inertia seen by the motor) and the torque needed during operation. This ultimately resulted in the use of a smaller motor (model BM8-2.5, Advanced Micro Systems Inc.) and since the motor was mounted directly on the printer head, a smaller motor resulted in a smaller payload, thereby improving the motion (*i.e.* greater acceleration/deceleration) of the printer head itself, a specification that was deemed important for our application. Moreover, such a small extrusion resolution is another advantage of the process since it can produce features requiring fine details.

A swivel joint was added between the plunger and the translation stage to account for misalignments between the syringe plunger and its barrel. One problem that was encountered during the initial printing tests was that hot sugar glass had a tendency to stick to the nozzle's surface due to the obtuse angle (118.3°) of the nozzle. A new custom made nozzle was manufactured with a sharper tip (42.6°) and resolved this issue. However, it was observed that the extruded sugar glass did not solidify quickly enough, resulting in saggy structures. Accelerating the sugar glass solidification was considered as a means of minimizing the deformation of the sugar glass after printing. Thus, an air cooling system, regulated at 7 psi and controlled by an electronic solenoid valve (Type 6027 - direct-acting 2/2 way plunger valve, Bürkert), was added, fastened to the support and activated during the extrusion.

2.2 Description of the printer improvements

In addition to the complete re-design and replacement of the printer head, other improvements were made to the printer to make it adequate for the desired application. The printer head was too tall to fit under the top plastic plate of the original printer; therefore, a new aluminum top plate was designed and manufactured. The original plate hindered extrusions as the printer head's movements in the Z-axis were restricted to under 50 mm of height. The new aluminum plate, shown in Figure 2, allowed the printer head to move up as high as 80 mm. This piece also eased access to the top of the printer head, which facilitated its assembly. In order to increase the accuracy of movements in the Z-direction, the two original screws on each side of the printer were removed and replaced with precision ACME lead screws (MTSBR8-500, Misumi).

2.3 Method used to print sugar glass constructs

The sugar glass mixture, used as the bulk material for our application, was prepared using 50 mL of water, 53 g of sucrose and 25 g of glucose. After dissolving the sucrose and glucose in water, the solution was heated on a hot plate (SCILOGEX MS7-H550-Pro) in a beaker to remove water and to produce the sugar glass syrup. Once the mixture's temperature reached 176°C, the beaker was removed from the hot plate. This bright yellow sugar mixture was then transferred into a preheated syringe to ensure that the material would not solidify upon contacting the syringe surfaces. To do this, the nozzle was removed and a plastic tube was connected directly to the tip of the syringe. The plastic tube was dipped in the sugar mixture and the plunger was manually pulled to fill the syringe. The syringe was then inserted into the printer head, ready for extrusion. The syringe and its content were heated and kept at an elevated temperature (~110°C) to prevent the premature solidification of the bulk material (*i.e.* syrup) prior to extrusion. The water percentage by mass of the extruded samples was estimated to be below 6% based on the weight change after drying in an oven.

The modified AW3D XL printer is fully compatible with available software tools (Cura, Slic3r, Repetier-Host, MatterControl). Prior to every printing session, a G-code program was generated for manufacturing the desired construct. As with conventional FDM printing, the G-code program can be hand-written or generated using a commercially available software. Both approaches were used, depending on the geometry to be printed. Repetier-Host freeware was used since it offered all the tools needed to write, simulate and send any G-code command to the printer.

2.4 Fabrication of vascularized constructs using rapid casting

To establish proof-of-concept of the method illustrated in Figure 1 without using cells, polydimethylsiloxane (PDMS) was used as the gel matrix during the rapid casting. Once the sugar glass vascular networks were 3D printed, PDMS (SYLGARD 184, Dow Corning) was prepared in a 10:1 ratio and placed under a vacuum at 28 mmHg for approximately 15 minutes to remove bubbles. The PDMS was then cast around the sugar glass network and put under a vacuum for a second time. The construct was then cured at room temperature

for 48 hours, after which the sugar glass was dissolved in water at room temperature. Complete sugar dissolution was achieved within 30 minutes under agitation (BlotBoy™, Benchmark Scientific).

Similar constructs were produced using a softer agarose hydrogel instead of PDMS. Prior to casting the agarose (UltraPure LMP Agarose, Life Technologies), the sugar glass construct was coated with poly(lactide-*co*-glycolide) (PLGA) to prevent premature sugar dissolution in the hydrogel. The sugar glass networks were immersed in a 25 mg/mL solution of PLGA (Sigma) in chloroform for 5 minutes. The PLGA solution was then removed and the construct was left for a few minutes under the hood to allow any excess chloroform to evaporate prior to casting the agarose. Once the agarose was poured around the coated sugar glass network, it was allowed to solidify for 10 minutes at room temperature, after which the sugar glass was dissolved and removed.

Preliminary perfusion tests with the cell-free PDMS and agarose "vascularized" constructs were performed using a peristaltic pump and 3-way valve connected to two reservoirs, one of which contained water and the other one colored water.

3. Results and discussion

3.1 Printer characterization

Once the sugar glass preparation procedure was established, the printing method was refined by testing different printing conditions to obtain accurate 3D structures. The feed rate (*i.e.* the printer head displacement speed) and the extrusion rate have a large impact on the fabrication process. A fast feed rate (*e.g.* 7 mm/s) combined with a slow extrusion rate (*e.g.* 1 mm/s) leads to a smaller filament diameter since the filament is stretched out of the nozzle during the printing. The effect of these two parameters on the size of the extruded filaments was characterized to select suitable printing conditions for producing variable-diameter structures. Based on the principle of mass conservation, we hypothesized that the diameter of the construct would obey the following relation:

$$d = C \cdot \left(\frac{v_e}{v_f}\right)^{.5} + b \qquad (1)$$

where v_e is the extrusion rate, v_f is the feed rate, C is a constant that takes into account the diameter of the nozzle as well as variations due to the contraction of the material during cooling (*i.e.* change in density during the process), and b is an offset parameter that takes into account various uncertainties in the model (*e.g.* uncertainties associated with the extrusion and feed rates, as well as the underlying assumptions such as mass losses due to evaporation). Since the diameter of the nozzle was 1 mm, it was expected that the value of parameter C would be close to unity and that the value of parameter b would be close to 0. In order to validate this model and to determine the values of C and b, three series of prints were performed at different extrusion rates (20 mm/min, 35 mm/min and 50 mm/min). For each extrusion rate, different feed rates ranging from 4 mm/min to 140 mm/min were used, and for each condition, 7 prints were produced. The diameter of each construct was measured at 3 points along the sample and the average diameter was used in the model. Standard deviations ranged from 0.02 mm to 0.05 mm, which is small relative to the range of diameters obtained (0.3 mm to 3.2 mm). The results are presented in Figure 3. In Figure 3a, the results have been linearized so that a linear regression could be performed to determine the values of C and b. These values were found to be 1.025 ± 0.007 and -0.08 ± 0.01, respectively, which are close to the expected values

(R^2 = 0.996). In Figure 3b, it can be clearly seen that when the feed rate is greater than the extrusion rate, the filaments are stretched, forming smaller diameters.

3.2 3D printed sugar glass test models

The new sugar glass printer head made it possible to produce long, self-supporting structures, as can be seen in the supplementary video A1 (see Appendix A). To test and demonstrate the full potential of the printer head, several 3D structures were manufactured. The first test model, presented in Figure 4a, is the Stanford Bunny, made famous by Greg Turk and Marc Levoy in 1993. This model has been used extensively in computer graphics [28] and was used to test the 3D printing process since it presents challenges such as the long overhang of the ears. The model was first imported as a CAD file, then scaled to be 53 mm high and sliced into 0.5 mm layers, resulting in a total of 106 layers. The Stanford Bunny was successfully 3D printed with sugar glass as shown in Figure 4b using a 1 mm nozzle. The chosen Z-resolution (0.5 mm) reduced the accuracy of the features that could be produced and explains the lack of fine features seen in the sugar glass model. Figure 4c shows the sugar glass Bunny 2 weeks post-printing, and it was observed that the surfaces were smoother even though the construct was kept in a low-humidity enclosure. Figure 4d shows the sugar glass Bunny 1 month post-printing, and it can be clearly seen that the surfaces of the construction continued to smoothen. Furthermore, the entire construct began to sag, a reminder of the temporary nature of the sugar glass material.

The second model that was printed to test the 3D printing process was a statue of Sir Wilfrid Laurier (1841-1919), seventh Prime Minister of Canada and a McGill University alumnus. The design of this statue has been utilized in prior research performed by Barnett and Gosselin [29, 30] on weak support material techniques and large-scale 3D printing using a cable-suspended robot. Barnett and Gosselin graciously provided us with the PLY rendering (Figure 4f) and the STL files for the statue design produced at the Computer Vision and Systems Laboratory of Laval University. The virtual model was obtained using a Creaform Go!Scan 3D scanner and the original statue (Figure 4e), created by Louis-Philippe Hébert in 1898, was loaned by the Museum Collections of Laval University. Barnett and Gosselin also provided us with a photo of the large-scale

3D printed replica of the statue they created using foam deposition (Figure 4g). Figure 4h shows the Laurier statue obtained using sugar glass 3D printing. The sugar glass statue was 78 mm high and was printed in 156 layers of 0.5 mm with the 1 mm custom nozzle. Once again, some fine features could not be printed owing to the chosen 0.5 mm Z-resolution. This model presents features that are very difficult to 3D print, such as the horizontal hemline of Laurier's coat. This feature was successfully printed with sugar glass without any supporting material due to the outstanding self-supporting behaviour of the material. To our knowledge, no other material is able to perform such long overhang features.

3.3 Temporary 3D printed sugar glass structures and associated constructs obtained by rapid casting

The next task undertaken was the creation of a 3D structure mimicking a vascular network. This was accomplished by performing the steps outlined in Figure 1, but without adding any living cells and using polydimethylsiloxane (PDMS) as the gel matrix in order to demonstrate the proof of concept. Figure 5 illustrates the steps performed to obtain the final PDMS construct. First, a G-code program was written to obtain the desired geometry (Figure 5a). The geometry was then printed in a standard 100 mm Petri dish (Figure 5b). Integrated into the design was an external rectangular sugar glass border, serving as a mould into which the PDMS could be cast. The air cooling system, previously described, was used during the sugar glass printing process and resulted in much more accurate prints. Prior to casting the PDMS, unwanted sugar glass filaments initially required to support the structure during the 3D printing process were removed manually (Figures 5c-d). Figures 5e-f show the sugar glass structure before and after casting the PDMS. The final PDMS construct (Figure 5g) was then injected with colored water to visualize the hollow network of channels (Figure 5h). Due to the orthogonal geometry of the channels, air bubbles occluded some of the channels and remained trapped in the junctions, preventing a complete perfusion of the construct. It was observed that much of the liquid flowed through the central channel, which offered less resistance to the flow.

Other vascularized PDMS constructs were produced using self-supporting sugar glass structures (Figure 6). This eliminated the need for the filament trimming step described in Figures 5c-d, allowing for gel casting immediately after 3D printing. Manual perfusion of a single-channel PDMS construct (Figure 6c) was

successfully performed using a syringe filled with red colored water and Luer lock needles (gauge 20) connected directly to the inlet and outlet of the construct. In Figure 6d, a 3-channel PDMS construct was perfused with colored water using a peristaltic pump. Figure 7 presents a series snapshots taken at 1-second intervals from the video A2 provided as Supplementary material. As show in Figure 7 and in video A2, the fluid flow was uniform and all channels were successfully perfused. Minor leakage was observed at the inlet and outlet of the construct. This issue may be resolved by adjusting the size of the connectors to better fit the channels, by modifying the geometry of the input and output channels, as well as by adding a sealant, such as additional PDMS cast around the connectors. Finally, Figures 6e-f show a 4-channel PDMS construct before and after sugar glass dissolution. Self-supporting horizontal, vertical and arching filaments can be easily produced and connected together in order to produce a complex vascular network. Moreover, the extrusion and feed rates can be dynamically changed within the same printing session, yielding filament diameters ranging from 0.3 mm to 3.2 mm without changing the 1-mm nozzle (Figure 3). This suggests that small and complex vascular networks may be rapidly produced using this technique.

Subsequent to the PDMS experiments, vascularized constructs were prepared using an agarose gel matrix more suitable for 3D cell culture. Figure 8 shows a 3-channel agarose construct connected within a perfusion loop. While all channels were successfully perfused without occlusion, greater leakage was observed at the inlet and outlet of the construct when compared with the perfused PDMS constructs. This is because the agarose gel was less stiff than the PDMS, and the connectors did not sit as tightly in the channels. Future work will therefore aim to modify the perfusion setup to avoid leakage when using softer hydrogels. A potential solution is to design a sample holder containing built-in connectors. The hydrogel would then be cast directly into this sample holder, creating a much tighter seal around the connectors. These agarose perfusion results are a promising indicator that this technique for 3D printing vasculature may be applied to 3D cell culture systems.

4. Perspectives and concluding remarks

A new 3D printer head was successfully developed and utilized for the production of temporary sugar glass structures. With this system, various types of structures were generated, including test models such as the Stanford Bunny. The developed technique was inspired by pioneers like Bellan *et al.* [31] and Miller *et al.* [25] but presents several advantages compared to previous work. A major improvement is the independent and precise control over parameters such as the syringe/nozzle temperatures and the cooling system, which establishes a printing process in which the sugar glass solidifies immediately upon extrusion. This allows for the production of self-supporting structures ideal for rapid casting, and also confers the ability to print in any direction along the x-, y- and z-axes. Accurate control over the extrusion rate, owing to the syringe-driven design, results in a better control over the filament diameter. Moreover, it was shown that a proper combination of extrusion and feed rates could be used to manufacture varying diameters within a single construct. This is another major improvement upon previous work, in which filament diameter was controlled by the displacement speed of the printer head. The new design is capable of printing larger filament diameters, thereby offering new possibilities for the creation of complex vasculature and reducing vascular patency issues.

Perfusable PDMS constructs were successfully produced using 3D printed sugar glass networks. This suggests an alternative avenue for the fabrication of 3D microfluidic networks for lab-on-a-chip applications. The perfusion of an agarose construct was also achieved, demonstrating that this technique is applicable to hydrogel-based systems, which are commonly used for 3D cell culture, tissue engineering, and cell therapy devices. An example of a possible application for this sugar glass printing system is the fabrication of a vascularized encapsulation device to deliver pancreatic beta cells to type 1 diabetics. The use of 3D printed sugar glass networks to create artificial vasculature could enhance the oxygen and nutrient distribution within the device, thereby improving cell performance. With this method, a variety of hydrogels could be used, including alginate, which is a commonly used biomaterial for pancreatic beta cell encapsulation.

Similarly, other bioartificial tissue constructs containing vasculature may be generated, such as cardiac patches for cardiac tissue repair and bone grafts for skeletal tissue repair. This could play an important role in overcoming oxygen limitations and facilitating the translation of *in vitro* tissue engineered therapeutic models to clinical success. Another potential application for sugar glass printing is the development of flow models to study cardiovascular disease. For example, aneurysm models may be produced by printing vessels exhibiting the "ballooning" characteristic of the disease. Studying fluid dynamics within such a model could further an understanding of disease pathogenesis.

The process that was developed could also be used for non-tissue engineering applications. For example, it could be used as a tool to produce easily dissolvable structures in more conventional 3D printing applications or to produce decorative designs for the food industry. The process could also be adapted for use with other materials, such as those dissolved in a solvent, or even with chocolate to produce custom, on-demand and complex pieces.

Utilizing additive manufacturing techniques in biomedical applications is a new paradigm that could be a game changer in the lives of millions of people. The work presented in this paper offers a possible route for developing a commercial sugar glass printer suitable for a variety of applications in the field of regenerative medicine. The process, design and results described herein constitute a promising step towards engineering vascularized bioartificial tissues.

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Appendix A – Supplementary material

Supplementary data associated with this article can be found, in the online version, at:

A1: sugar glass 3D printing

A2: perfusion of 3 channel construct

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Figure captions

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

Figure 2: New printer design: a) computer-aided design (CAD) of the motorized translation stage, b) CAD of the new printer head, c) final printer head once all the parts were manufactured and assembled, d) CAD view of the modified Airwolf3D XL (AW3DXL) printer with new sugar glass printing head, e) photo of the modified printer after the printing of the Stanford Bunny.

Figure 3: Average filament diameters obtained at 3 different extrusion rates (20 mm/min, 35 mm/min and 50 mm/min) and at different feed rates (ranging from 4 mm/min to 140 mm/min): a) average diameter plotted against the square root of the ratio of the extrusion rate over the feed rate and b) average diameter plotted against the ratio of the feed rate over the extrusion rate. The model fit was significant in both cases ($p < 0.0001$).

Figure 4: Stanford Bunny and Sir Wilfrid Laurier test models: a) Original Stanford Bunny (courtesy of Stanford Computer Graphics Laboratory [18]), b) 3D printed sugar glass Stanford Bunny produced using the process described in this paper (106 layers of 0.5 mm), c) Bunny 2 weeks post-printing, d) Bunny 1 month post-printing, e) Sir Wilfrid Laurier original plaster statue (courtesy of Museum Collections of Laval University), f) PLY file rendering, g) 3D printed foam statue (courtesy of Barnett and Gosselin, Laboratoire de Robotique de l'Université Laval [30]), h) sugar glass statue 3D printed with the new printer head (156 layers of 0.5 mm).

Figure 5: Production of a "vascularized" PDMS construct: a) virtual representation of the desired channels, b) sugar glass structure during the 3D printing process, c) top view of the sugar glass structure, including inlet and outlet ports and an integrated exterior mould, d) top view of the sugar glass structure after sugar glass support filaments were removed, e) isometric view of the temporary sugar glass structure before casting PDMS, f) sugar glass structure after casting PDMS, g) PDMS construct after sugar glass removal, h) PDMS construct with red colored water injected into the channels.

Figure 6: Various "vascularized" PDMS constructs produced by rapid casting using sugar glass: a) single channel PDMS construct, b) 3-channel PDMS construct prior to dissolution of the sugar glass, c) manual perfusion of a single-channel PDMS construct, d) pump perfusion of a 3-channel PDMS construct (See Supplementary Video A2), e) 4-channel temporary sugar glass network before casting PDMS, f) 4-channel PDMS construct after sugar glass dissolution.

Figure 7: Series of pictures taken at 1-second interval showing the transition for clear water to colored water during the perfusion of the 3-channel PDMS construct; numbers indicate the time elapse in seconds since the beginning of the transition (see Supplementary Video A2).

Figure 8: 3-channel “vascularized” agarose construct produced by rapid casting using 3D printed sugar glass and connected to a peristaltic pump prior to perfusion.