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DIRECT BIOPRINTING OF ALGINATE-BASED TUBULAR CONSTRUCTS USING MULTI-NOZZLE EXTRUSION-BASED TECHNIQUE

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ABSTRACT: Bioprinting is a layer-by-layer additive fabrication technique for making three-dimensional (3D) tissue and organ constructs using biological products. In this study, alginate tubes of varying viscosity are being printed, which mimic typical tracheal tube, are fabricated vertically using extrusion based technique. In addition, manufacturing challenges associated with the vertical printing configurations are briefly discussed. This study lays a foundation for the successful fabrication of viable 3D tubular constructs.

Introduction

Bioprinting, a rapidly emerging technology in specifically assembling both living and non-living biological materials into its ideal complex layout, has been termed as a possible new paradigm as it requires a solid-scaffold free process for constructing tissue (Guillemot, Mironov, & Nakamura, 2010). Thus, with this in mind, researchers in bioprinting mostly involved a mixture of hydrogels and cells during printing. However, one of the major drawbacks in using hydrogel during cell printing is the viscosity of the gel(Kong, 2003). If the viscosity of the gel is too high it would cause cell death due to the high shearing during the extrusion process. If the viscosity of the gel is too low, the shape fidelity of the construct would be not be retained after printing. One of the ways to overcome such dilemma would be to complete the gelation after printing. Although some research has been done on cross-linking alginate gels simultaneously during printing but those has been relatively slower as they are done in the solution or on the surface of the solution(Changxue Xu, Yong Huang, 2012). Thus these methods have to account for surface tension and the speed of the extrusion to get a good resolution.

The main objective of this study is to investigate the feasibility in fabricating 3D vertical tubular structure using a multi-nozzle extrusion based technique to process low viscosity hydrogel. The resulting knowledge gained in this preliminary study will help to enable future study in tubular designs for tissue engineering.

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Materials and Methods

Hydrogel

Sodium alginate powder (Sigma-Aldrich) was dissolved at 0.06g/ml in PBS solution under constant stirring. Then 0.01g/ml to 0.03g/ml of Xanthan gum from *Xanthomonas campestris* (Sigma-Aldrich) was dissolved into the alginate solution. Then 0.022g/ml of calcium chloride (Sigma-Aldrich) is added dropwise to the mixture. Additionally 500mM of Calcium chloride was prepared as an additional cross-linking solution. The hydrogel is then placed into a time-based extruder while calcium chloride is placed in a microvalved controlled dispenser.

Hydrogel Characterisation

Rheology of the hydrogel was characterized using a Discovery Hybrid Rheometer 2 (TA Instruments) using a 40 mm parallel plate geometery with a measurement gap of 0.5 mm and Peltier plate thermal control. After loading, the samples were conditioned by subjecting to 30s pre-shear at $500s^{-1}$ followed by a 1 min equilibrium before measurements were taken. Shear-dependent viscosity was evaluated using a stepped ramp of shear rate from $1\text{-}1000s^{-1}$. Measurements were taken at 10 points per decade.

Printing Process

Tubular structure design and process was input into the bioprinter using BioCad (RegenHu). First, a circular structure of radius of 6mm was defined in the system as the extrusion route for the hydrogel. Then a secondary loop of radius of 4mm which is concentric with the hydrogel path was made for the dispensing route for calcium chloride. The pressure of the bioprinter is set at 1.5bar for the hydrogel and 0.5bar for calcium chloride. Tubular constructs were printed using RegenHu's Biofactory. Hydrogel was extruded through a 0.25mm syringe needle (Needle DD-135N-N4) while the $CaCl_2$ solution is dispense through a 0.3mm needle tip. The path speed of the hydrogel is 500mm/min while the path speed of the $CaCl_2$ is 100mm/min and the valve is open for 2Hz. $CaCl_2$ solution's path speed was much lower than the hydrogel to allow it to sufficiently fill up the tube during dispensing. The layer thickness of the hydrogel is set at 0.2mm. To allow time for the layers to fuse before allowing the gel to cross-link, the printer was programmed to dispense $CaCl_2$ only after 3 layers has been built. Once the gel and $CaCl_2$ interacts, further gelation occurs providing it sufficient structural integrity to hold on the next few layers of gel. The process of printing is shown in figure 1. To demonstrate the feasibility of the print, the structure was printed to a height of 4.8mm.

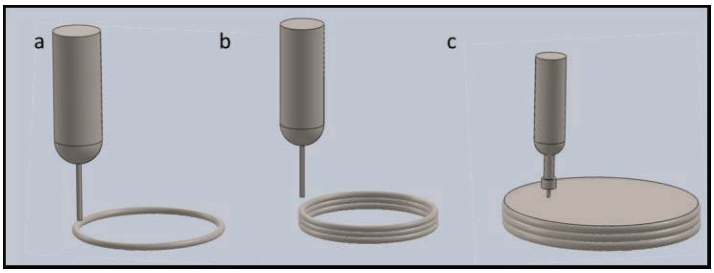

Figure 1: Schematic diagram of printing process of Alginate-Xanthan gum gel (a) Extruding hydrogel first layer of gel, (b) extruding 3 layers of gel, (c) filling the tubular construct with $CaCl_2$ solution

Results and discussion

In this study, Xanthan gum was used to determine the optimal viscosity for alginate to retain its shape fidelity after printing. Xanthan gum is an anionic polysaccharide produced by the bacterium *Xanthomonas Capestris*(Iseki, Takahashi, Hattori, Hatakeyama, & Hatakeyama, 2001). The Xanthan gum has been used in multiple applications ranging from food, agriculture, petroleum and pharmaceutical industry. It is used mainly as viscosity enhancer and stabilizer in blends and has been reported to contain bioadhesive properties(Jaipal, Pandey, Abhishek, Vinay, & Charde, 2013). Also since Xanthan gum has only weak interaction with $CaCl_2$(Sworn & Biopolymers, 2009), it makes a good filler and will have low interactions in affecting the results. To understand how viscose the gel is and its shear thinning properties, the hydrogel was characterized using a rheometer and the results are reported in Figure 2. With increasing amounts of Xanthan gum, the solution tends towards a more viscous behavior. This viscosity will affect the amount of gel extruded and the overall printing fidelity of the construct.

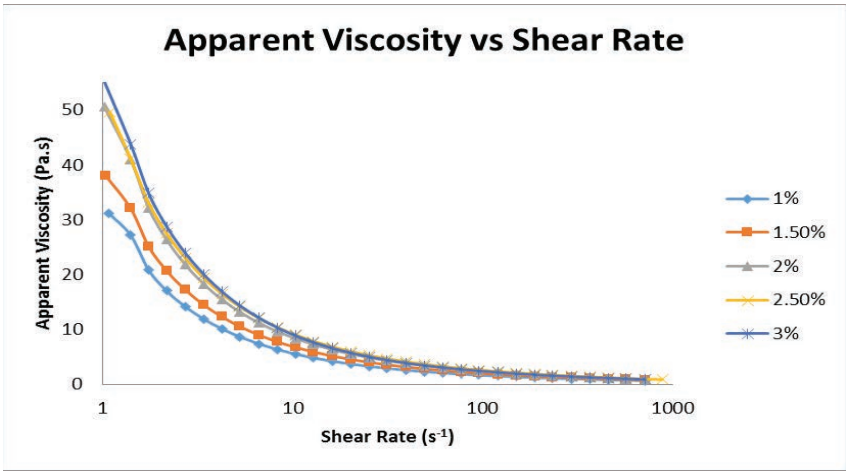

Figure 2: Viscosity as a function of shear rate for Alginate-Xanthan Gum Gel. Xanthan Gum concentration was varied from 1% (blue diamond), 1.5% (orange square), 2% (grey triangle), 2.5% (yellow cross) and 3% (blue asterisk).

The photos of the printed construct are shown in Figure 3. When the Xanthan gum used is too low (1%), we can see that there is an overall spreading of the hydrogel as compared to the rest of the construct. Additionally, we can also see that in e, the gel was too viscous and the gel was not printed appropriately. This can be seen from the defects and cave-in seen in figure 2j. In Figure 3 b,c,d and e, we can see layering effects on the hydrogel similar to those constructs built used in most additive manufacturing techniques. From the results, it seems that 2% xanthan gum may seem to be produce the best print with more perpendicular growth in thickness compared to the rest of the constructs.

All the construct has an opaque interior due to the cross-linking effect of $CaCl_2$ on the Alginate Xanthan gum gel, creating a gradient of mechanical properties radially towards the outer wall of the gel wall.

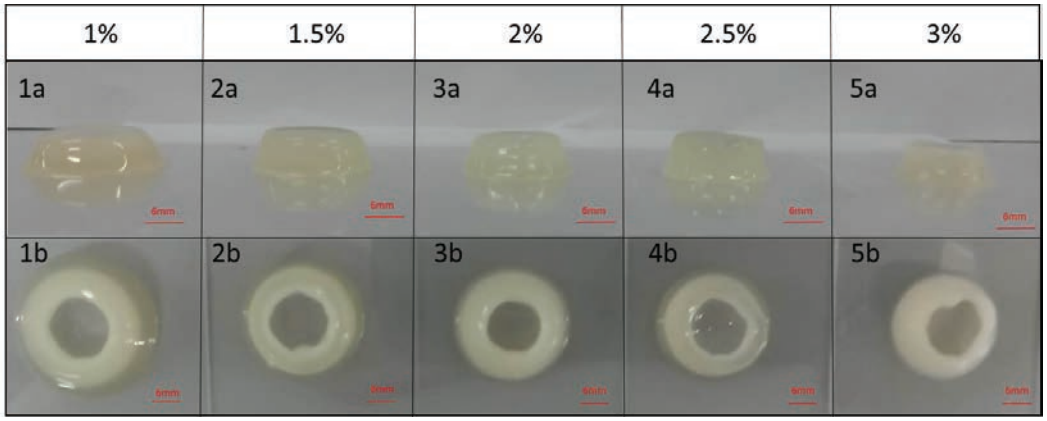

Figure 3: Photographs of alginate-xanthan gum gel tube of (a & e) 1% xanthan gum, (b & f) 1.5% xanthan gum, (c & h) 2% xanthan gum, (d & i) 2.5% xanthan gum and (e & j) 3% xanthan gum. a-e represent the front view of the xanthan gum gel while f-j represent the top view of the tube structure. (Scale bar: 5mm)

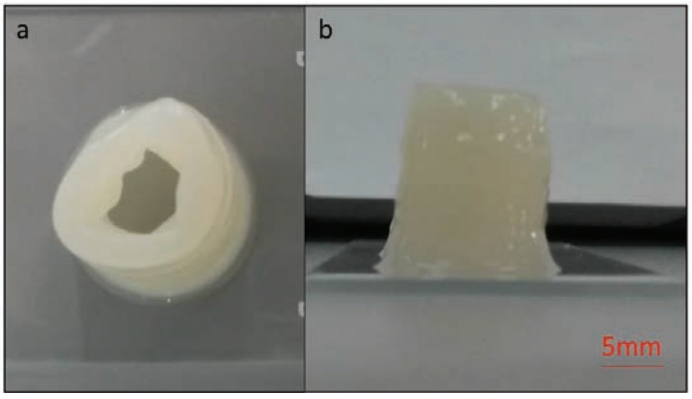

Figure 4: Photographs of 15mm tubular 2% Xanthan Gum Alginate Gel (a) top view of the tube structure and (b) the front view of the Xanthan gum gel. (Scale bar: 5mm)

To show that the construct is feasible to withstand higher structural load, another printed construct of 15mm was fabricated using the 2% Xanthan gum-Alginate gel as shown in figure 4. Interestingly, the structure was relatively uniform in width between each layers above the 3-4mm mark, thus giving less variations in width as compared to the lower layers. Compared to previous print, there seems to be a more evident shrinkage effect caused by the $CaCl_2$ gelation interaction. This shrinkage could be resolved by using a low concentration of $CaCl_2$. However, the tube is still relatively straight with little deviation from its designated shape.

Conclusion

The tubular printing capability was discussed in this paper and it has shown that tubular constructs have been successful printed by controlling the viscosity of the gel and through the use of a new fabrication technique to dispense $CaCl_2$ directly into the tube. This process of printing was able to create a gradient of mechanical properties as the crosslinking effect was more dominant within the gel. The process of printing tubular structure was also able to allow printing of taller structures. However, the shrinkage caused by cross-linking of $CaCl_2$ has to be resolved before the tubular structure is able to be printed higher and better resolution. This process of printing is still in its infancy and many of the parameters still have to be optimized before a perfect result can be produced. However, as such, the potential of this process could possibly improve the current problem in balancing shape fidelity and cell viability during printing. Future work in this research could include the printing of branching tubes which current casting methods have failed to achieve and possibly computational models which can determine the optimal parameters and design for printing.

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