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UNDERSTANDING THE RELATION BETWEEN PRINTING AND MATERIAL PARAMETERS OF TIME-PRESSURE DISPENSING SYSTEM FOR BIOPRINTING

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ABSTRACT: Bioprinting, a computer-aided printing, is an emerging field in tissue engineering. The ability to print biological models from scanned medical images allows customizability in printed construct. Identifying input printing factors that affects dimensions of printed biological construct is important as it enables control over geometry of structure. Biocompatible hydrogel is used in bioprinting to incorporate cells into printing process. In this experiment, a time-pressure dispensing system is used. This article investigates different factors such as pressure of system, nozzle inner diameter, moving speed, and viscoelastic material that define the dimensions of printed construct. It is identified printed hydrogel achieves finer resolution at higher concentration, faster moving speed, smaller nozzle inner diameter and lower pressure.

INTRODUCTION

Tissue engineering aims to provide engineering solutions to medical problems. Three elements are present in the field of tissue engineering i) cells ii) biomaterials iii) bioactive factors. Of which, biomaterials provide the structural support for cells to grow and proliferate. Different fabrication methods have been explored to shape biomaterials into support structures for cells. Conventionally, biomaterials are formed through solvent casting, solution casting, electrospinning, etc. A major limitation to these fabrication methods is the inability to control geometry of the printed construct. These structures lack uniform pore size and pore distribution (Ferris, Gilmore, Wallace, & Panhuis, 2013). Thus, in recent years, computer-assisted system has been used to construct defined solid scaffolds for cells to reside in.

Additive Manufacturing uses computer-assisted system to print three dimensional physical models layer-by-layer from digital data. AM system allows control over geometry of construct and has been adapted to tissue engineering. With AM technology, solid scaffold has been printed using fused deposition modeling (Tellis et al., 2008), selective laser sintering (Williams et al., 2005), selective laser melting (Fukuda et al., 2011), and stereolithography (Melchels, Feijen, & Grijpma, 2009). Printed solid scaffolds are followed by cell seeding except for metal scaffolds that are directly implanted into patients. One of the challenges to cell seeding is the non-uniformity is cell distribution within the construct. Therefore, an alternative is to incorporate cells into printing system.

Several printing methods have been explored to include cells during printing. Cell printing processes can be broadly classified into three categories namely i) extrusion ii) inkjet iii) laser-based techniques (Ozolat & Yu, 2013). While both inkjet and laser-based printing has reported printing with precision dispensing close to single-cell resolution (Abeyewickreme, Kwok, McEwan, & Jayasinghe, 2009; Guillotin et al., 2010), extrusion-based printing has the ability to

extrude materials that range from paste with high viscosity to solution with low viscosity. Moreover, cell aggregates can be printed through extrusion-based techniques (Jakab et al., 2010). A major difference between extrusion-based printing such as FDM with extrusion-based bioprinting is the processing condition used. In FDM, thermoplastic polymers are melted and pushed along two rollers to deposit polymeric filament onto platform. On the other hand, extrusion-based bioprinting deposit solution/gel based polymers either by piston or pneumatic pump. As such printing parameters may be different between extrusion-based FDM and bioprinting.

Extrusion systems have been widely used in the electronics manufacturing industry. Materials such as adhesives and epoxy have been deposited in lines or dots onto electronic chips. Several modeling methods have been developed to understand flow behavior and side profile of extruded material. These flow modeling systems can be integrated to provide a feedback loop system. This allows tuning of material output according to printing parameters that may change through time. Several parameters have been investigated and can be broken down into printing and material parameters. However, most time-pressure dispensing system used for electronics manufacturing comprises of a solenoid valve (Chen, Li, & Ding, 2007; Zhao, Li, Ding, & Xiong, 2005). The duration of open and close solenoid valve affects whether material dispensed is a line or dot. As such, this article will adopt and investigate printing and material parameters that are related to bioprinting.

Printing parameters such as stage speed, pressure and nozzle inner diameter are investigated in this article. Additionally, viscoelastic property of printing material is evaluated.

METHODS

All materials are purchased from Sigma Aldrich unless otherwise stated.

Preparation of Hydrogel

Two different concentrations of alginate hydrogel were prepared. The compositions are as shown in Table 1. Sodium alginate powder was weighed, dissolved in deionized water, and stirred using magnetic stirrer for 15 minutes. Alginate hydrogel was cross-linked before loading into syringe cartridge for experiment. As such, 1.5mL of calcium chloride was added drop-wise into 3.5mL of sodium alginate solution. A homogenizer was used to ensure uniform mixing between the two solutions. Thereafter, cross-linked hydrogel was loaded into 5mL syringe for printing.

Table 1 Concentration of alginate used

	Sodium Alginate (%wt)	Calcium Chloride (%wt)
High Concentration	6	2
Low Concentration	6	1

Rheological Property of Hydrogel

Hydrogel viscosity was measured using a parallel plate rheometer (DHR, TA Instruments, USA). A 40 mm diameter parallel plate measuring geometry with gap 500 μ m was used to perform an amplitude sweep under oscillatory mode. Constant frequency of 1Hz was set with logarithmic

increase in stress ranging of 0.001 – 100 Pa. Storage modulus (G') and loss modulus (G'') was recorded for three samples from each concentration.

Printing Parameters

Time-pressure dispensing system (Biofactory®, RegenHu, Germany) was used as the printing system for characterization. Pressure was set at 0.2 and 1 bar with stage speed varied between 500 and 800 mm min⁻¹. Two different nozzle sizes, 0.33 and 0.2mm, were used.

Quantifying printed construct

Three horizontal lines were drawn using BioCAD software available with the printing system. Width of printed construct was quantified using ImageJ software. A total of six measurements were taken for each printed parameter set up.

Design of Experiment

Four perimeters were explored to investigate on the effect of factors on printed construct. The four factors are alginate:calcium chloride concentration, pressure of printing system, speed of platform and inner diameter of nozzle. Eight runs were performed as according to Table 2.

Table 2 Setup of experiment with eight runs to investigate four factors

Parameters	+	-	A	B	C	D
	(High)	(Low)	[Conc]	[Pressure]	[Stage Speed]	[Inner Diameter]
%wt Concentration (Alg:Ca)	6:2	6:1	-	-	-	-
			+	-	-	+
Pressure (MPa)	0.3	0.2	-	+	-	+
			+	+	-	-
Moving Speed (mm/min)	800	500	-	-	+	+
			+	-	+	-
Nozzle Inner Diameter (mm)	0.33	0.2	-	+	+	-
			+	+	+	+

RESULTS AND DISCUSSION

Storage modulus, loss modulus and complex shear viscosity of alginate hydrogel with varying calcium chloride concentration was measured using DHR rheometer. Figure 1 displays the result from rheometric measurement. Viscosity profile obtained display a Non-Newtonian (Shear Thinning) fluid flow behavior. Alginate hydrogel that contains higher concentration of calcium content displayed less steep curve as compared to hydrogel with lower calcium concentration. Similarly, Farres et al has reported a similar trend in alginate hydrogel with varying alginate: calcium ratio (Farres, Douaire, & Norton, 2013).

Figure 1 a) Modulus of varying alginate: calcium chloride concentration b) viscosity profile

Storage modulus varied between high and low calcium content alginate hydrogel as seen in Figure 1. This can be explained by the increase in the calcium ions present with higher concentration of calcium chloride. As calcium ions act as the crosslinking agent in alginate hydrogel, an increase in the concentration of calcium ions yield alginate hydrogel with higher cross-linked polymer chains.

Figure 2 Comparison graphs between two factors a) Inner Diameter VS Moving Speed b) Moving Speed VS Pressure c) Concentration of hydrogel VS Moving Speed

From Figure 2, a few observations can be made regarding achieving constructs with finer width. Small construct width can be achieved using high concentration alginate hydrogel, high moving

speed, small nozzle inner diameter and low pressure. As displayed in Figure 1, alginate hydrogel with higher concentration has a higher viscoelastic property. Rheometric property of hydrogel can be correlated to the amount of material being dispensed. This can be explained using a simple non-newtonian volume flow rate equation that relates flow rate with viscosity of hydrogel as displayed through Equation 1 to 4 below.

Power law model can be used to approximate viscosity data

$$\eta = \eta_0 \left(\frac{\dot{\gamma}}{\dot{\gamma}_0} \right)^{n-1} \quad \text{----- (1)}$$

where n is the power law index, η_0 is the zero viscosity, $\dot{\gamma}$ and $\dot{\gamma}_0$ are the shear rate and zero shear rate respectively. To identify flow rate Q of non-newtonian fluid in a simple cylindrical geometry, velocity profile of fluid has to be determine.

$$V_0 = K \left[\frac{n}{n+1} \left(R \frac{n+1}{n} \right) \right] \quad \text{where } K \text{ is a function of } \frac{1}{\eta_0} \quad \text{----- (2)}$$

$$Q = \left(\frac{n+1}{3n+1} \right) \pi r^2 V_0 \quad \text{----- (3)}$$

$$\text{Volume} = \int Q \, dt \quad \text{----- (4)}$$

Velocity profile of fluid shown in Equation 2 explains that with higher viscosity fluid, the velocity profile will be lower. As described in Equation 3, flow rate of fluid is directly proportional to fluid's velocity. Since higher viscosity material develops lower velocity profile, the fluid has a lower flow rate. This resulted in lower volume of material extruded. Hence, it is explained that with high concentration of alginate hydrogel, construct's width becomes smaller. Figure 3 below show the comparison between runs with different factors. Figure 3b shows the camera image of construct with higher concentration alginate hydrogel, faster moving speed, smaller nozzle inner diameter and lower pressure. As seen from below, changed parameters results in constructs having smaller width and can be imaged under inverted microscope Figure 3c.

Figure 3 Camera (a and b) and inverted microscope (c) images of constructs with varying parameters a) Construct at 6:1 %wt concentration of Alginate:Ca hydrogel, 0.3MPa, 500mm/s Moving Speed, 0.33mm Nozzle Inner Diameter b) Construct at 6:2 %wt concentration of Alginate:Ca hydrogel, 0.02MPa, 1400mm/s Moving Speed, 0.2mm Nozzle Inner Diameter c) Inverted microscope image of b, Scale bar 1000 micron

CONCLUSION

This paper investigated printing parameters of a time-pressure bioprinting system and material property of a biocompatible hydrogel. Alginate hydrogel was used to understand the viscoelastic effect of material on printing resolution. It is found that with increased concentration of calcium chloride content, alginate hydrogel appears more solid-like due to an increase in crosslinking density. Several factors contribute to construct with smaller width. By changing factors such as higher concentration alginate hydrogel, faster moving speed, smaller nozzle inner diameter and

lower pressure, printing lines can achieve reduced width size. This study has investigated printing and material parameters at two levels. Future studies will require evaluation of each factor at more than two levels to identify any non-linear relationship between factors.

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