Journal of Physics: Conference Series

Effect of pressure and infill density parameter setting on morphological and mechanical properties of polycaprolactone printed scaffold using desktop 3D bioprinter

Effect of pressure and infill density parameter setting on morphological and mechanical properties of polycaprolactone printed scaffold using desktop 3D bioprinter

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Abstract. Three dimensional (3D) bioprinting is the process of building a 3D construct containing biological cells. There are varies set of printing parameter involved in 3D bioprinting system that will affect the structure and the mechanical properties of the bioprinted construct. This study was done to investigated the effect of extrusion pressure and infill density on the polycaprolactone (PCL) 3D printed construct's morphology, printing accuracy and compressive strength. Based on morphological evaluations using scanning electron microscope, the printed scaffold was suggested to be at the best condition at pressure 60psi using 80% infill setting. Besides, the mechanical testing revealed the printed scaffold was successfully fabricated without losing its mechanical integrity. This is a good indication for PCL as structural host material to obtain functionalized 3D scaffold using bioprinting method.

1. Introduction

Bioprinting is the process of dispensing cell-laden biomaterials for the construction of 3D functional living tissues or artificial organs [1]. On other hand, 3D printing technique also has been primarily used to create acellular 3D scaffolds and molds which then can be seeded with cells post-fabrication [2]. Material used to print in 3D bioprinting process such as combination of living biological cells, polymers, chemical factors, and biomolecules are known as bioinks [3]. There are three major methods of 3D bioprinting technologies that commonly used which are inkjet printing, laser-assisted printing, and extrusion printing [1].

Extrusion based bioprinting basically used a high air pressure (pneumatic) or mechanical force to dispense bioinks, by applying a non-stop force so that the extrusion printing can be printed uninterrupted rather than a single bioink droplet [1, 4]. Through this method, the resolution of 200 µm can be achieved, but compared to laser-based or inkjet-based this is considerably low [4]. In this system, the bioinks are required to be compatible where it has suitable rheological properties and crosslinking mechanisms to enable accurate and precise deposition. The material should be sufficiently viscous to be dispensed as a free standing filament and it must have sufficient strength and stiffness to maintain structural integrity after printing.

Polycaprolactone (PCL) is a semi crystalline polymer with about -60 °C for its glass transition temperature [5]. With just at point 58 to 60°C, PCL can be melt down, making it suitable with other polymers [6, 7]. Compared to polylactic acid (PLA), the degradation rate for PCL is much lower, thus make it valuable base polymers for a long term drug delivery devices. Besides, PCL possessed a good rheological and viscoelestic property among resorbable polymers which make it suitable candidate to be studied.

In bioprinting process, there are several parameter that need to be consider such as pressure, shear stress, temperature, feed rate, dispensing speed, and nozzle diameter [4]. All these parameter will affect the 3D printed construct structure and also their mechanical properties. Currently, there are limited studies focus on the accuracy of printing technique using this PCL. Here, this study aim to test the printability that is the ability of material to be accurately and precisely deposited with desired degree of spatial and temporal control. The morphology and mechanical properties of printed scaffold will be investigated as indication for best parameter printing. This study is important as it can be served as guideline to print 3D construct using PCL with high accuracy.

2. Methodology

2.1. Printing process
PCL (BioBots) in solid beads form, white in color and odorless was used in the printing process to generate 3D scaffold. A dual layered 9.9 mm x 9.9 mm x 1.8 mm grid was designed by using CAD software (SolidWorks). Then the .stl file was converted to .gcode file by loaded into repetier host (Slic3r) to adjust the preferred printing parameters. 5 mg of PCL were filled into metal syringe capped with 30 gauge metal needle. The design 3D construct was printed by using commercial desktop 3D bioprinter (Biobots, USA) at 100°C to allow the PCL to melt. The extrusion occurs at layer height of 0.05 mm and printing speed was set to 1 mm/s. The extrusion pressure was set at 60 psi, 80 psi and 100 psi with infill density percentage of 60%, 80% and 100%. The printed scaffolds then were analyzed using scanning electron microscope and compression test.

2.2. Scanning electron microscope
The post-print morphology and the geometry structure of the printed scaffold were observed using tabletop scanning electron microscope (SEM) (Hitachi TM3000) at 50X magnification and further analyzed by using imageJ software.

2.3. Accuracy
The SEM micrographs were analyzed using ImageJ to find dimensions of the printed area. The printed area, A_i (mm^2) was compared to the design area, A (mm^2). The printing accuracy for each sample was calculated using following equation, with average of three grids used.

$$\text{Printing accuracy (\%)} = \left[1 - \frac{(A_i - A)}{A}\right] \times 100 \qquad (1)$$

2.4. Compression test
Uniaxial compression tests were performed using universal tensile machine (INSTRON 5566) at a loading rate 0.1 mm/s with a load 10N load cell. The upper and lower sample surfaces were fixed to the platens of the tester to ensure there was no slippage during tests. For each test, three samples were used and their average values calculated.

3. Results and discussion

Microscopic examinations were carried out on the printed scaffolds at different pressure and infill density percentage. From the SEM image of the upper view of the printed construct, the construct shows the porous structure which indicates it is suitable for cell attachment (Figure 1a) [8]. Apart from that, the strand thickness also were observed and measured from SEM image using imageJ. The strand thicknesses of extruded PCL scaffold through 30G needle are shown in Table 1. The printed construct print with 60 psi and 80 % infill density produced the thinnest strand (1.25 mm) where printed construct print with 100 psi and 100 % infill density produced the thickest strand (1.53mm). It can be seen that at same infill density percentage, the increasing extrusion pressure will produced thicker strand width construct resulting from high extrusion pressure forces the PCL through the needle gauge. Action of gravity also might have an impact to the PCL ink as it will caused an increase in width, making pooling inevitable [9]. Therefore, there is a limit to the minimum achievable strand thickness.

The printing accuracy was calculated using previous mentioned formula (1). Results show that increasing the extrusion pressure with fixed infill density percentage results in less accurate printing (Figure 2). However, printing with increasing infill density with fixed extrusion pressure does not produce any specific pattern for the printing accuracy. Printing with 60 psi of extrusion pressure and 80 % infill density will produce highest accuracy (88.78%) of 3D printed construct. According to Di *et al.*, the final geometric accuracy is a combination of many factors, including the effects of depositing materials in successive layers [10]. Here, this study shows the effect of extrusion pressure on multilayered grid dimension and found that accuracy reduces with printing in increasing extrusion pressure. The compressive modulus was found highest (17.8 MPa) when printing at low pressure of 60 psi with 80% infill density percentage (Table 1).

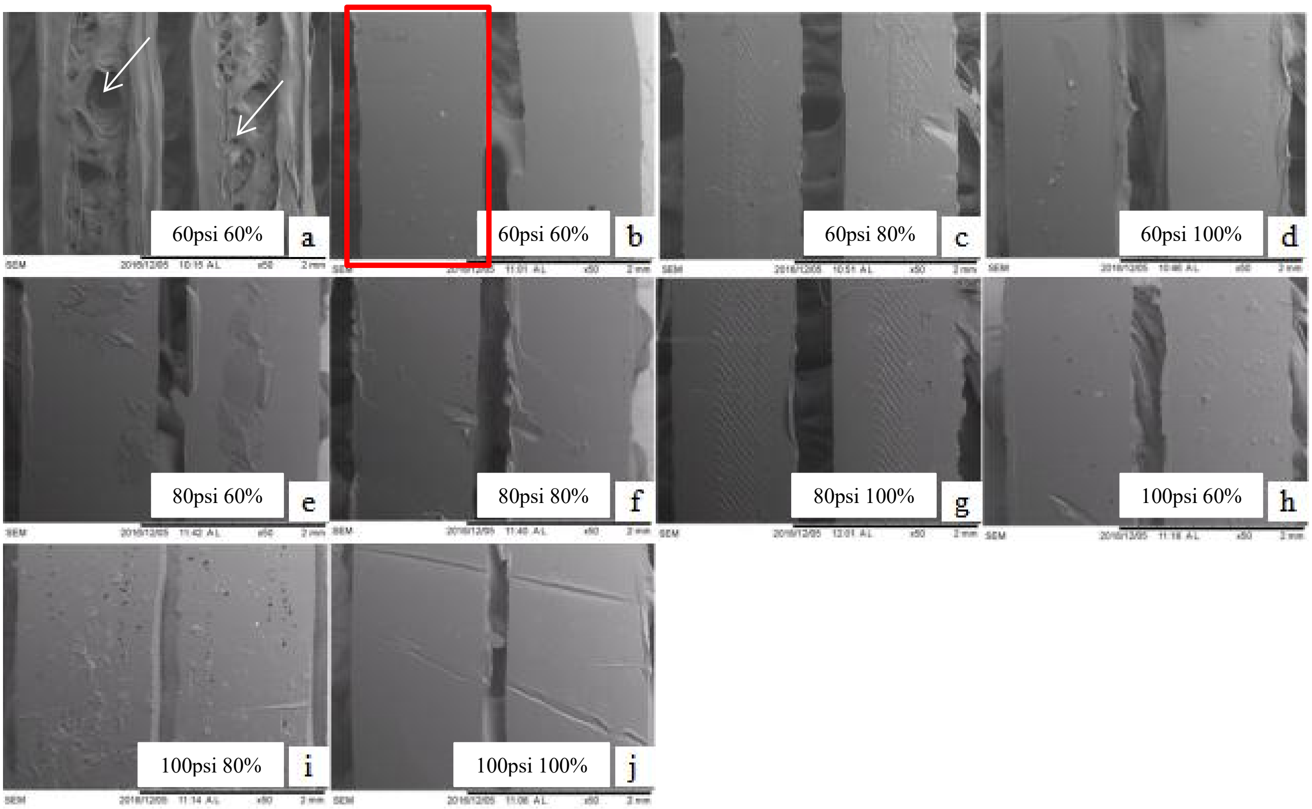

Figure 1 SEM image of the 3D printed construct. An upper view of the printed construct with porous structure showed with arrow (a), bottom view of printed construct, different strand (red box) thickness produced (b-j).

Table 1 Strand width, printing accuracy and compressive modulus of the 3D printed construct

Pressure (psi)	Infill Density (%)	Strand Width (mm)	Printing Accuracy (%)	Compressive Modulus (MPa)
60	60	1.31	87.12	17.1
	80	1.25	88.78	17.8
	100	1.35	88.26	16.3
80	60	1.43	86.74	16.1
	80	1.39	87.04	16.7
	100	1.39	87.64	16.3
100	60	1.41	86.72	16.3
	80	1.49	85.88	15.9
	100	1.53	85.99	16.5

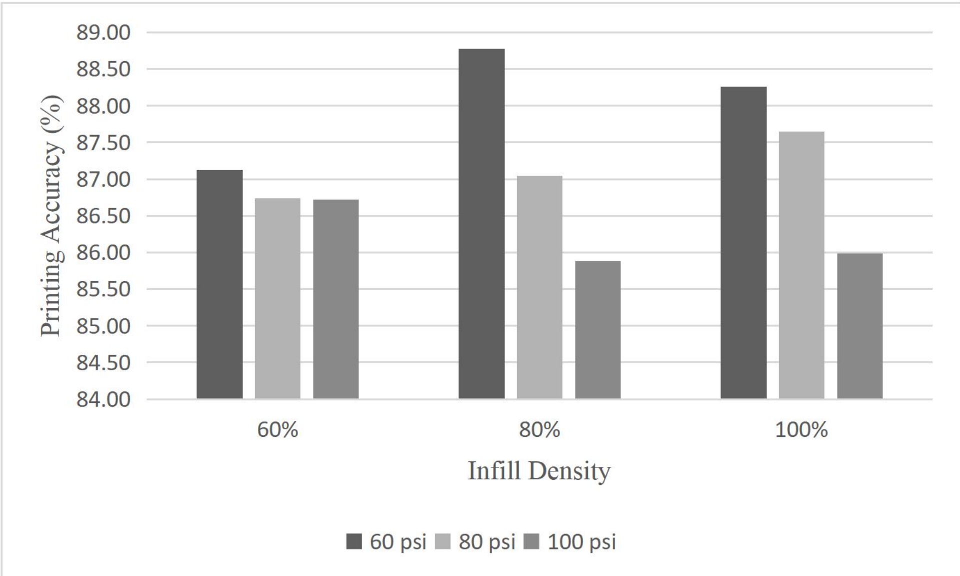

Figure 2 Printing accuracy of 3D printed construct

4. Conclusion

Herein, this study have presented and described the effect of extrusion pressure and infill density on the structure, print accuracy and mechanical properties of the printed construct. Based on the result, the best parameters achieve to fabricate dual layer PCL scaffold were at pressure 60psi and by using infill density of 80% where it produce construct with highest printing accuracy and compressive strength. From the value obtained, it can be concluded that the parameter successfully fabricate the scaffold construct with a good flow and without losing its mechanical strength.

Acknowledgment
The authors would like to acknowledge Ministry of Higher Education Malaysia for funding through Fundamental Research Grant Scheme.

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