Analysis of 3D-Printed Vascular Scaffold At Varying Extruder Temperature And Flow Rate

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Accepted December 2020, available online 30 December 2020

ABSTRACT

Three dimensional (3D) vascular scaffolds architecture must meet the requirements such as biocompatibility, biodegradability and high porosity. One of the important mechanical properties is the scaffold porosity that is related the pore size. Besides mechanical properties, scaffold porosity also can influence the biological properties such as cell attachment, cell proliferation, and cell differentiation. Meanwhile, 3D printing techniques have been used widely in producing customized scaffolds from the microscale to the macroscale by using a layer by layer approach. Eventually, 3D printing parameters play an important role in determining the pore size of the printed scaffold. Therefore, by using polylactic acid (PLA), here we investigated the effect of temperature and flow rate on 3D-printed scaffold to determine the optimum combined parameter in producing a vascular scaffold with the desired porosity properties. In this study, the best combined parameter was 230°C as temperature and 100 mm/s as the printing speed to enable the 3D printer to replicate a high accuracy 3D scaffold.

Keywords: Tissue engineering, vascular scaffold, 3D-printed, extruder, temperature, flow rate

1. Introduction

The vascular system is the most complex and vital network in the human body that is not only critical for nutrient transferring, but also involved in several tissue homeostasis such as wound healing, bone remodelling and cancer metastases [1]. However, in engineered tissue, the supply of oxygen and nutrients is limited with 150–200 μm of the total thickness of cultivated tissue [1]. Therefore, current vascular replacement for vascular disease is highly limited due to the complicated of the blood vessel itself. Vascular graft can be made from natural matrix, synthetic polymers, or other biological materials [2]. New approach had been introduced by vascular tissue engineering by developing a synthetic functional graft with morphological, mechanical and biological properties similar those of native vessels known as vascular graft [3]. Artificial grafts represent a concrete solution for the replacement of large and medium diameter vessels solving the present limitations of autologous vessels use [4]. Clinically approved vascular prostheses are increasingly suitable as alternatives to autologous arterial and venous vascular replacements due to high mortality and impairment, due to cardiovascular disease [5]. For therapy, tissue vasculature design must be highly vascularized or promote the formation of new vasculatures at the replace site [6]. On the other hand, existing vascular scaffold are lack control over physical and mechanical properties and others biological properties such inflammation [7].

Ideally, the architecture of 3D scaffolds must meet the requirements for the vascular scaffolding such as biocompatibility, biodegradability and high porosity. Besides adequate mechanical properties, the scaffold also need to have suitable structure for cell seeding with high similarity with native extracellular matrix to support tissue regeneration. Porous 3D scaffolds are commonly since the porosity of the scaffold influenced cell attachment, cell proliferation, and cell differentiation [8]. Therefore, high porosity materials are preferable in allowing the successful release of biofactors as well as good nutrient exchange [9]. Interconnected pores of the porous surface have increase biocompatibility and enable more vascular ingrowth and incorporation [10]. However, the pore size needs to be large enough for the indirect contact of cell across the scaffold since large pores effected the cell adhesion properties [11].

Tissue engineering involves the use of solid and porous matrices scaffolds that enable the development of a new tissue by providing mechanical stability and improving the cell morphology [12]. Various methods has been developed to fabricate 3D porous scaffolds such as particle leaching, freeze drying, or foaming, have limitations to precisely control the pore structures, and the pore-templating agents are easy to remain [13]. Recently, 3D printing techniques have been used widely with the advantages in producing customized scaffolds. 3D printing has been widely used in tissue fabrication with a structural control from the microscale to the macroscale by using a layer by layer approach. 3D printing able to construct a biomimetic structural environment that facilitates tissue formation and promotes host tissue integration such as cellular infiltration, vascularization and active remodeling [14]. 3D printing is a rapid prototyping technique for the manufacture of micro-scale porous structures of the desired complexities, which enables the true engineering of the scaffold.

In 3D printing, viscous materials are extruded in a layer-by-layer fashion from fine nozzles by compressed air to form strands and porous scaffolds [13]. In this context, poly(lactic acid) (PLA) has attracted the attention of the scientific community due to its biocompatibility, biodegradability, ease of processing and thermal stability. PLA is known as one of the most extensively used synthetic polymers due to its non-toxicity, thermoplastic, high strength and high modulus polymer [15]. Besides, PLA is widely used in the manufacture of vascular grafts due to its biocompatibility and biodegradability [16]. There, 3D printing parameters play an important role in determining the optimal condition of structure of vascular scaffold. Therefore, by using polylactic acid (PLA), here we investigated the effect of temperature and flow rate on 3D-printed scaffold to determine the optimum parameter in producing a vascular scaffold with the desired porosity properties

2. Methodology

2.1 Materials

Poly Lactic Acid (PLA) was used as the material for 3D printing. PLA as a bioplastic is its versatility and the fact that it naturally degrades when exposed to the environment. The optimal printing PLA temperature range from about 185°C to about 205°C as it may increase depending on the thickness of PLA filament and the condition of the 3D printer itself

2.2 3D-Printed Scaffold Fabrication

The low cost 3D filament printer (MakerBot 2X) was used to print the scaffold. Using a computer-aided design (CAD) software package (SolidWorks 2013), a solid 3D scaffold of the required length, width and height was designed. 3D CAD modeling was exported as a. STL mesh file format for production using 3D printer software (Makerware 2.4.1, MakerBot). The method was used to make 'woodpile ' as scaffolds in the x and y directions with regularly spaced aligned polymer filaments. Scaffold printing parameter were carried out to find the optimal parameter that has the smallest error when compared with the original design. The printing parameters that were studied were extruder temperature (ET) and fluid flow rate (FR). The recommended extruder temperature was from 210ºC until 230ºC. This was because any temperature that lower from 210ºC will make the filament stuck at the nozzle of 3D printer as there are not enough heat to melt the filament and if the temperature above 230ºC will damage the filament. Meanwhile the fluid flow rate was 100 mm/s until 150 mm/s.

Table 1. Scaffold printing parameter

Sample	1	2	3	4	5
Extruder temperature (°C)	210	220	230	230	230
Fluid flow rate (mm/s)	150	150	150	130	100

After that, the printed vascular Scaffold structure were analyzed by Inverted Fluorescent Microscope (ZEISS) attached to a digital camera by using Zen Blue Software for the collection and scale setting of digital images. These images were processed using the Image J software.

3. Results and Discussions

The main intention behind the scaffold structure study was to visualize the relationship between the printing process parameters with the end product of the original design scaffolds. Figure 1(a) shows the isometric view where it has dimensional of 20.1mm diameter and 4.8mm height with circle shape. The measurement of the pores located on surface have length and width of 0.3mm or 300µm, as shown in Figure 1(b).

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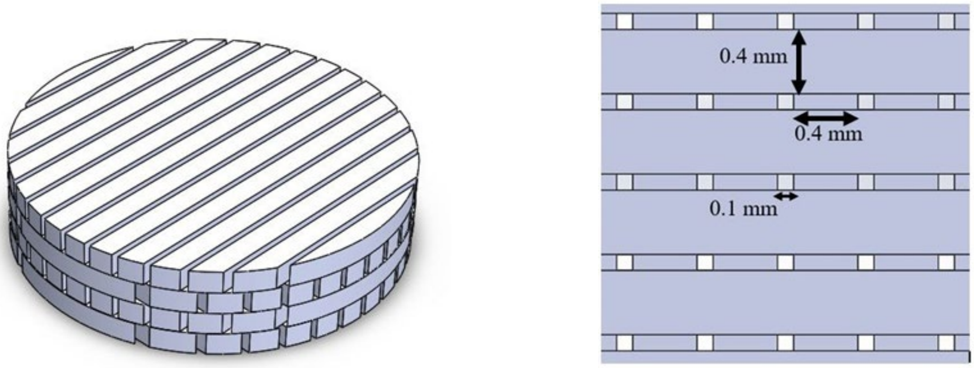

(a) (b)

Figure 1. (a) Isometric view of the scaffold; (b) pore dimension of the scaffold

. The layer of surface was stacked on top of each other and pores are been extruded in order to have continuous pipeline of pores. Therefore, the area of the pore is 90000 μm^2. The distance between filaments was 0.1 mm and with 4 layers. Figure 2 shows the representative image of printed PLA scaffold.

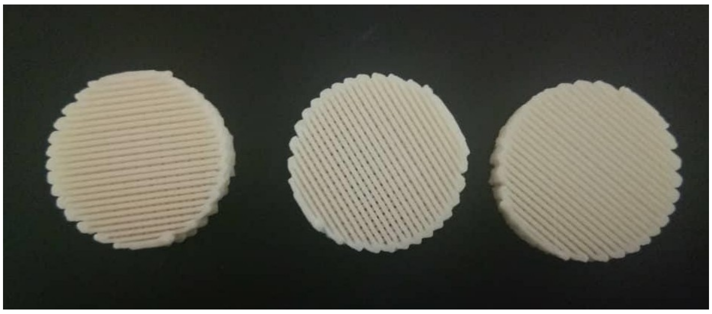

Figure 2. Fabricated Vascular Scaffold

The 3D printed vascular scaffolds were observed under an inverted fluorescent microscope to observe the scaffold pores. Three images were taken with at least three pores on it for each 5 different scaffold parameters. Figure 3 shows the three representative images that were captured for scaffolds that were printed with 250°C temperature and 150mm/s printing speed. The length and height from the captured images were measured to compare with drawing design, which was designed as 300µm.

(a) (b) (c)

Figure 3. Representative image of the scaffold captured by fluorescent microscope

The fifteen images that have been captured by the ZEISS microscope were measured using ImageJ software. Image J software measured the length and the width of the pores for every image taken. The original area of the pores for scaffold design in SolidWorks was compared with the pores for the printed scaffold design. From the ImageJ software analysis, the length of the pores was measured to compare with the original pore length. As for the result, the average pore length for five different setting of temperature and printing speed has been obtained as shown in Table 2.

Table 2. Average pore length and pore height at different parameter

Parameter (°C, mm/s)	**Length (µm)**	**Height (µm)**	**Area (µm²⁾**
210, 150	353.7	320.9	113530.1
220,150	277.6	167.5	46505.1
230,150	319.5	225.9	72161.8
230,130	308.9	165.4	51095.1
230,100	291.3	264.7	77091.2

As compared to original design, which was 300µm for both pore length and pore height, the printing version shown the variation based on the printer parameter setting. From Table 2, it was clear that printing at 230°C and 130 mm/s gave most accurate pore length, which was 308.9µm. Meanwhile, printing at 210°C and 150 mm/s gave the most accurate pore height, which was 320.9 µm. Table 2 also shows that printing at 210°C and 150 mm/s gave the highest calculated area which was 113530.1µm^2 and printing at 220°C and 150 mm/s gave the lowest calculated area, which was 46505.1 µm^2. Nevertheless, Table 3 shows the percentage error of area of scaffold pore for each combined parameter setting.

Table 3. Percentage error of area of 3D printed scaffold pore

Parameter (°C, mm/s)	**Percentage error**
210, 150	26.14%
220,150	48.33%
230,150	19.82%
230,130	43.23%
230,100	14.34%

From the calculation, the measured area at five different settings of printing temperature and printing speed shows 3D printed scaffold has 14% to 48% percentage error compared to the area of original pore size that was designed using SolidWorks. For temperature, the least error percentage error was at 230°C which was optimum and suitable temperature for 3D printing. For printing speed, the least percentage error was at 100mm/s. By comparing these results, it shows that combination 230°C temperature and 100 mm/s printing speed was the best optimum parameter with the lowest error for the calculated area. Previous study shows that the increase of temperature, flow rate, and printing speed has the effect in increasing the filament width of scaffold during 3D Printing [17]. However, the temperature of phase separation did not have a clear effect on the porosity. The findings show that the pore size decreased as the temperature decreased, although the nanofibers' diameter did not change significantly with the temperature [17]. At the same polymer concentration, the direction of the radial temperature gradient did not significantly affect the average pore size [17]. A study suggested that adhesive strength of filament increased with the flow rate and this was closely related to travel speed of 3D printer which was defined as the speed of extruder travels along the horizontal plane [18]. Another study concluded that 3D temperature was going to affect particles emission and quality of printing [19]. Meanwhile, the scaffold with woodpile infill pattern has been proved to support homogeneous cell growth [20]. Moreover, oriented tubular pores design in scaffold provide an easier pathway for cell seeding and uniform distribution throughout the scaffold [21]. The scaffolds 3D pore structure and surface morphology affect the quality of the tissue formation on the scaffold.

4. Conclusions

In conclusion, the best setting for the 3D printer to be able to replicate the 3D model dimensions was 230°C as the platform temperature and 100 mm/s as the printing speed. It was proven through this project that to reproduce results and trends from other research within the same field of 3D manufactured medical solutions, the process of selecting the right materials and parameters was incredibly crucial. Therefore, 3D printing has high potential in implementing into vascular engineering solutions as it allows customizability of scaffold designs and production processes.

Acknowledgment

The authors would like to acknowledge Universiti Teknologi Malaysia for funding through Research University Grant Scheme Tier 2 (Q.J130000.2651.17J03.

5. References

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