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3-dimensional printing of PLA scaffolds for medical applications

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ABSTRACT

Scaffolds encourage the new tissue formation through biological substitution of the damaged or lost tissues. Therefore, scaffold characteristics become more important and should be precisely controlled. Production of scaffolds using a three dimensional (3d) printer appears as a promising method in terms of enabling homogeneous pore distribution and uniform pore size arrangement. In this study, polylactic acid (PLA) scaffold structures were obtained through 3d printing, based on the design parameters such as the scaffold geometry, porosity (%), pore shape, pore size, and the pore interconnectivity. An open source computer-aided design (CAD) program (Interface Scaffold) was employed to design the PLA scaffolds. Scaffold structures with ~72% porosity were generated through a 3D Systems Cube 2nd Generation 3d printer. The design parameters have been optimized by the scaffold design software tool, which includes different unit cells, i.e. Schwartz P, Schwartz D, Gyroid, Skeletal (1-4), Neovius and W (iWP) for designing scaffold structures through mathematical formulations. It was found out that the mean pore size of the 3d-printed Gyroid unit cell scaffolds vary between 1.9 mm and ~4.54 mm according to the microstructural observations done by a scanning electron microscope (SEM).

1. INTRODUCTION

Three dimensional (3d) printing systems play a significant role in fulfilling the demand for biomedical applications. It is possible to enable better anatomic compatibility between the defect area and 3d-printed patient-specific implant materials so that a better healing process can be managed. Complicated shaped implant materials can be designed and produced one-to-one coherent to the damaged tissue such as bone fractures by using the computed tomography (CT) scan and rapid prototyping systems collaboration. In this context, researchers pay considerable attention to the porous materials, which are frequently utilized as bone scaffolds in hard tissue implants. Porous implants not only reduce the elastic (E) modulus of the material but also ensure superior osseointegration with the bone tissue and the implant material by facilitating the migration of the bone cells through the pores. An interconnected pore network on the implant surface provide potential surface area for the cells to adhere, grow and proliferate. The increased surface area owing to the porous structure of the material enhances the biological interactions between the material and the bone tissue so that osseointegration is promoted. Besides, the decreased E-modulus allows the implant material and the bone tissue properly share the applied load compatible with the human anatomy; hence, stress shielding can be avoided [El-Hajje et al. 2014; Wang et al. 2016]. Since bone is a structure composed of parts with different mechanical strength, mimicking the E-modulus characteristics of the host tissue, i.e. spongy (cancellous) bone and cortical bone, is an inevitable condition for the implant materials that are employed for hard tissue treatments. Therefore, porous materials draw interest in terms of possessing E-modulus values close to that of the spongy bone. Porous structures improve the mechanical integrity between the implant material and tissue while accelerating the bone growth depending on the interconnected pore network that assures the continuous transition of the body fluids, nutrition, and oxygen towards the open pores. Thus, it should be emphasized that porous structures function for both better mechanical fixation and osseointegration [Wang et al. 2016; Ryan et al. 2008; Limmahakhun et al, 2017].

There are several factors that should be taken into consideration to design and prepare a convenient bone scaffold for the target hard tissue. Pore size, por shape,

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pore interconnectivity, porosity (%) and strut (cell wall) thickness are the essential criteria that should be elaborately designated before the production process [Van Bael et al. 2011; Kang et al. 2017]. Indeed, in order to realize rapid bone growth, porosity (%) amount should be in the range (~75-85%) that will enable the implant material to have an E-modulus value close to that of the spongy bone [Wally et al. 2015]. Pore size is reported to be larger than 100 μm for the scaffolds that are intended to be used for orthopedic applications to provide better biological interactions between the scaffold and bone tissue and therefore, rapid bone regeneration [Papadimitropoulos et al. 2007; Tarafder et al. 2013]. Recently, 3d printing has been widely implemented as an efficient way to produce scaffold structures with controlled pore size, porosity %, pore interconnectivity, strut thickness, etc. for biomedical applications [Wally et al. 2015; Bose et al. 2012]. As the scaffold structures are employed as the templates for tissue regeneration, scaffolds made of different materials such as metallic, ceramic, polymer or composite of them are extensively used in tissue engineering applications to promote the repairing process. From this point of view, it was aimed to design polylactic acid (PLA) scaffolds, which have suitable design characteristics to be the template candidates for regeneration.

PLA is a biocompatible material widely used in biomedical applications such as orthopedic implants/prostheses (pins, screws, sutures, synthetic constructs, plates, etc.), tissue engineering scaffolds, dental resin, drug delivery systems and theranostics, etc. PLA is defined as a biodegradable material, and the degradation products of PLA are not harmful to the body. This situation enables PLA to be utilized in the demands above [Navarro et al. 2005; Felfel et al. 2012; Senatov et al. 2016; Da Silva et al. 2018; DeStefano et al. 2020; Gendviliene et al. 2020; Wu et al. 2020]. Although PLA is well known for being bioresorbable, its mechanical properties limit the usage areas to non-load bearing applications. Therefore, a composite can be prepared, where PLA is reinforced by bioceramics like hydroxyapatite (HA) and bioactive glasses. Consequently, the mechanical strength could be increased, and osseointegration of PLA could be enhanced, as noted in the literature [Navarro et al. 2005; Serra et al. 2013; Senatov et al. 2016; DeStefano et al. 2020; Gendviliene et al. 2020; Wu et al. 2020]. These composites can be implemented for bone defects to provide bone regeneration. Moreover, since PLA is a biodegradable polymer material, it is commonly used as a fixation device like screws and plates (Felfel et al., 2012). It is aimed to overcome the stress shielding problem that arises when metallic materials are employed due to the mismatch between the E-modulus values of the metallic implant material and hard tissue. In fact, Stener et al. 2010 demonstrated in a study carried out on 77 patients that poly-L-lactic acid (PLLA) screws used in the tibia and femur showed similar performance as metal screws during tendon reconstruction [Da Silva et al. 2018].

PLA scaffolds were developed with a computer aided design (CAD) software and manufactured via 3d printing. Microstructure and porosity properties of the scaffolds were investigated with a scanning electron microscope (SEM) and the operated scaffold design software.

2. METHOD

2.1. 3d Printer & PLA Filament

Porous scaffold structures were produced by employing a 3D Systems Cube 2nd Generation 3d printer. This 3d printer (**Fig. 1**) works with the fused deposition modelling (FDM) principle. FDM technique is based on melting and then layer by layer deposition of a thermoplastic material [Ngo et al. 2018]. In this research, polylactic acid (PLA) -a recyclable biodegradable thermoplastic polymer material- is used as the precursor in filament form. Properties of the 3d printer and PLA starting material are given in **Table 1**. Scaffold structures were obtained taking into account the design parameters such as porosity (%), pore interconnections, strut thickness and pore shape. Firstly, the properties and design parameters of the scaffold structures were investigated through a detailed literature research. By comparing the previous studies [Wang et al. 2016; Bose et al. 2012; Leong et al. 2003; Holzapfel et al. 2013; Cheung et al. 2007; Khang 2017], the characteristics and design parameters of an ideal scaffold structure were determined. CAD design of the scaffold structures were realized by benefitting from the open source software tool, i.e. "*Interface Scaffold*" presented and reported by Dinis et al. 2014 and Castro et al. 2019.

2.2. Designing the Scaffold Structures

Scaffold structures can be designed with various CAD programs. Several researchers managed to design scaffolds by employing already existing CAD programs such as Solidworks [Wang et al. 2017], CASTS (for tissue engineering scaffolds) [Sudarmadji et al. 2012], Pro/Engineer [Naing et al. 2005], CATE [Nam et al. 2004] and MATLAB [Monkova et al. 2017]. Researchers have a tendency to create scaffolds by associating unit cell structures. Creating scaffold designs by combining the unit cells with different geometric shapes is pretty convenient. For example, Chantarapanich et al. 2012, designed scaffolds with a 119 polyhedron unit cell model in their work. Although CAD programs can be directly used for designing the scaffold structures, actually it can be occasionally difficult to realize such complicated designs. Scaffold structures may have sophisticated geometries and the complex design parameters may obstruct the possibility of a feasible design, especially for the researchers who newly begin creating scaffolds. Therefore, software programs, which form scaffold structures from unit cells can be evaluated as a remedy. Scaffold structures can be easily created by selecting the unit cell type and determining the design parameters with these scaffold designing software programs. Dinis et al. 2014 presented an open source software tool, which is named as "*Interface Scaffold*" for this purpose and the mentioned tool includes different unit cells, i.e. Schwartz P, Schwartz D, Gyroid, Skeletal (1-4), Neovius and W (iWP) for designing scaffold structures through mathematical formulations. Unit cells are converted to the scaffold structures according to the x-y-z dimensions and pore size values that are manually entered by the user [Dinis et al. 2014].

In the current work, Interface Scaffold software tool was used to design the scaffold structures. The user can individually decide the design parameters such as scaffold geometry, scaffold dimensions, porosity (%), pore size, pore shape, etc. utilizing the Interface Scaffold software tool (**Fig. 2**). A 15 x 15 x 30 mm *Gyroid* unit cell scaffold structure was designed via the Interface Scaffold software tool [Dinis et al. 2014; Castro et al. 2019]. Moreover, it can be stated that the Gyroid unit cell has a suitable morphology for tissue regeneration applications due to its superior pore interconnectivity [Khang 2017; Dinis et al. 2014; Castro et al. 2019].

Figure 1. Picture of the 3D Systems Cube 2nd generation 3d printer

Designs that are created with Interface Scaffold software tool can be saved directly in *stl* file type. Since the 3d printer functions with the stl files, the designs should be transferred to the 3d printer slicing programs. Hence, the design file was saved as a stl file. Then, the ready-to-print stl file was transferred to the Cube 2nd generation 3d printer's own *Cubify* software. Pre-printing parameter selections were done with this software. In this context, the position of the part on the tray, number of the layers, printing time, support structure usage (support and/or raft) and the raw material (PLA or ABS) adjustments were carried out (**Fig. 3**). Following the necessary adjustments, the production time of the scaffold structure was revealed as 48 min. by the Cubify software. According to the printing software, thickness of each molten PLA layer is maintained as 0.25 mm to enable the maximum printing resolution. The z length of the designed scaffold was determined as 32.25 mm (including the support parts) by the software. When the z length (32.25 mm) is divided to the layer thickness (0.25 mm), it is found out that the scaffold structure will be composed of 129 layers. This indicates that the 3d part will be ready after the sequential printing of 129 PLA layers.

Table 1. Features of the 3D Systems Cube 2nd Generation 3d Printer and polylactic acid (PLA) filament used for 3d printing [3D Systems 2014]

Property	Description/Value
Technology	Fused Deposition Modelling (FDM)
Max. print area	140 x 140 x 140 mm
Material	Durable thermoplastic polymers (ABS and PLA)
Layer thickness	250 µm
Support and raft	Fully automated
Cartridge	Roll filament
Filament diameter	1.75 mm
Nozzle operating temperature	280 °C
Adhesive for the printing tray	Water based adhesive

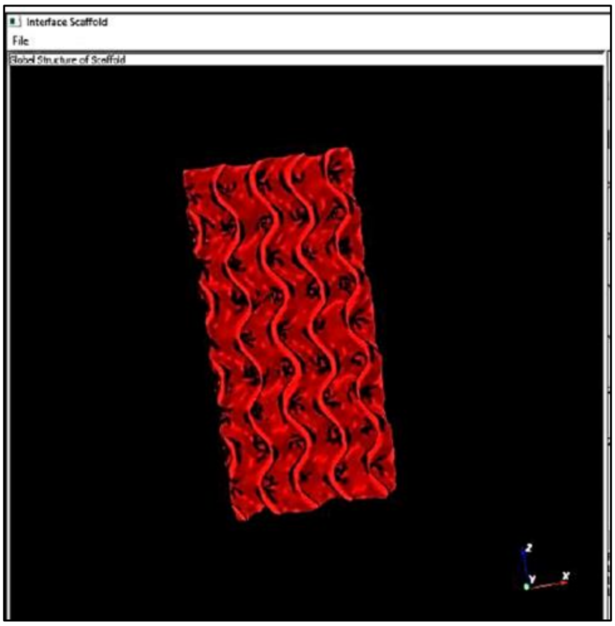

Figure 2. Design of the Gyroid unit cell scaffold structure

2.3. 3d Printing Process

The settings of the 3d printer has to be performed before each printing process. First, the thermoplastic filament (PLA) is fitted into the 3d printer. After selecting the new filament settings from the 3d printers' software, the print head automatically moves to the appropriate position. The small heater inside the 3d printer begins to heat up to 250 °C. The tip of the filament is then inserted through the hole that is placed on the top of the print head. The 3d printer transfers the filament to the heater by means of gear wheels. A little amount of filament may spill from the nozzle tip when the heater is completely filled with the material. However, this situation points out that the filament is properly connected to the printer. Then, the distance between the print head and the tray (z-axis) should be arranged. Since a fault in the z axis will disrupt the layers, this adjustment should be optimal. The 3d printer's print head and the tray automatically take the adjustment position while calibration is in progress. Afterwards, an A4 paper is placed between the nozzle tip and the tray. The nozzle tip and the tray are brought closer to each other by leaving a distance such that an A4 paper can move smoothly. When this implementation is completed, the 3d printer is ready to print. The filament placement and calibration of the 3d printer steps were repeated before each printing practice.

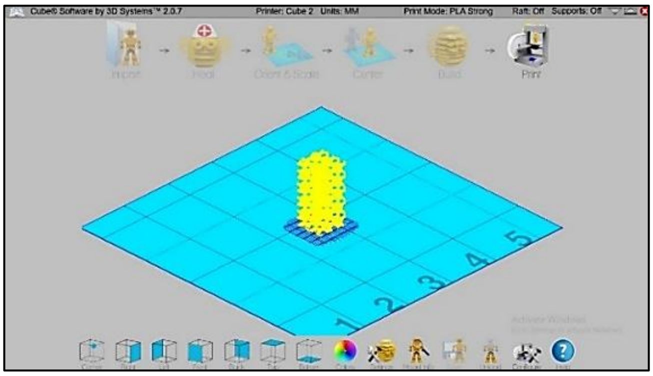

Figure 3. Ready-to-print version of the scaffold (Cubify software)

Within the 3d printing process (**Fig. 4**), primarily the water-based adhesive of the 3d printer was applied to the surface of the tray before printing starts. This adhesive allows the first molten filament layer to adhere to the tray surface. If the first layer does not adhere properly to the tray, then the following layers will be corrupted. As a result, the printing quality will decrease. The tray was removed from the 3d printer after the printing process was completed. The water-based adhesive of the 3d printer can be simply dissolved in water in approximately 5 min. so that the printed scaffold structure can be easily separated from the tray. The support parts of the scaffold structure can be extracted by applying slight mechanical impacts. The 3d printing process finished after separating the support parts and took totally 1 h and 5 min. including the pre-printing, printing (48 min.) and post-printing stages. Microstructural features of the 3d-printed PLA scaffolds were investigated with a FEI Quanta FEG 450 branded SEM and porosity value of the scaffolds was determined with the Interface Scaffold software tool.

Figure 4. Pictures of **(a)** the 3d printing process of the PLA scaffold and **(b)** the screen view of the 3d printer during the printing procedure

3. RESULTS & DISCUSSION

The 3d-printed porous scaffold structures were properly obtained by using the FDM method. It was macroscopically observed that the 3d-printed PLA scaffolds have a highly porous structure with superior interconnectivity and no deformities were recognized on the surface or general construction of the 3d-printed parts. It was proven that the actual dimensions (15 x 15 x 30 mm) of the 3d-printed PLA scaffolds are compatible with the CAD design created with the Interface Scaffold open source software tool (**Fig. 5**). The ellipsoid pore shape of the scaffolds can be realized from the SEM images **(Fig. 6)** of the top and lateral surfaces of the scaffolds. The 3d-printed scaffold structures possess a porosity of 72%, which was assigned by the employed scaffold design software tool. 72% porosity is appropriate for the tissue engineering applications, where the healing process is accelerated and osseointegration between the tissue and material is enhanced due to the highly porous structure of the scaffolds [Wang et al. 2016; Ryan et al. 2008; Limmahakhun et al. 2017; Papadimitropoulos et al. 2007; Tarafder et al. 2013]. The mean longitudinal size of the pores and the mean latitudinal size of the pores were measured as ~4.54 mm and ~1.9 mm from the SEM images **(Fig. 6)** of the top and lateral surfaces of the 3d-printed PLA parts by means of the digital ruler in the software of the SEM facility.

The SEM images in **Fig. 7** clearly indicate the layer-by-layer deposition of the fused PLA filaments around the pore walls. The 3d-printed layers have a pretty smooth surface despite the unattached tiny molten filament pieces. No gaps, voids or looseness between the molten PLA layers were detected, in spite of the highly porous structure of the part. This has demonstrated the effectiveness of the 3d-printing techniques in terms of generating components with complex geometries.

4. CONCLUSION

PLA is a biocompatible material widely used in biomedical applications and a popular material that can effectively function in 3d printing mechanisms. Recently, the production of PLA scaffolds with complicated structures for different medical demands such as orthopedic implants or tissue engineering studies has become more attractive. Design parameters of the scaffold structures are very important for the target biomedical application. Scaffolds with complex morphologies and more accurate characteristics like porosity (%), pore interconnectivity, etc. have been produced by employing 3d printing as a new generation manufacturing method. Since the design parameters influence the application area of the scaffold structures, they should be considered carefully. At this point, 3d printing offers a precise control of the design parameters. In the present report, Gyroid unit cell PLA scaffolds, which were one-to-one compatible with the CAD data, were practically obtained by using a 3d printer that works based on the FDM principle.

Figure 5. Pictures from different surfaces of the 3d-printed PLA scaffolds

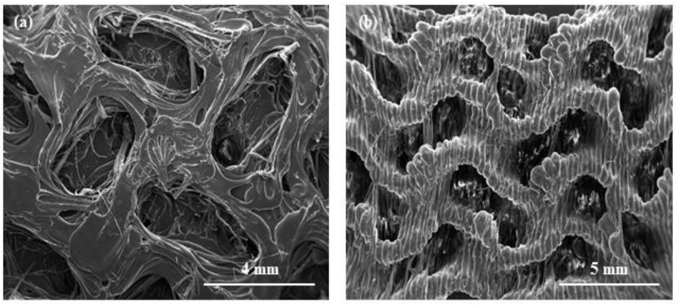

Figure 6. SEM images of the 3d-printed PLA scaffolds (**a:** top surface, **b:** lateral surface)

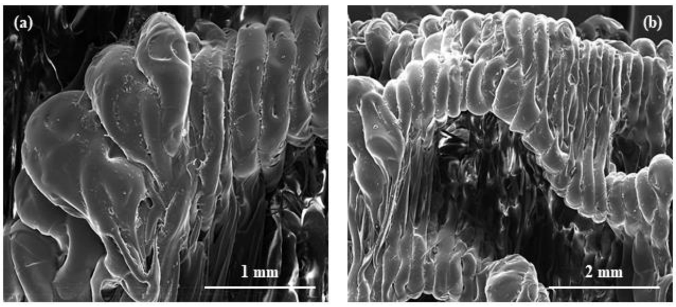

Figure 7. SEM images of the pore walls of the 3d-printed PLA scaffolds

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Author contributions

Azade Yelten: Determining the aim and creating the methodology of the study, Analyzing the characterization data, Writing-Reviewing and Editing the manuscript. **Mehmet Halit Öztürk:** Designing the Scaffold Structures, Printing Process, Help with the preparation of the manuscript draft. **Suat Yılmaz:** Coordinating the study.

Conflicts of interest

The authors declare no conflicts of interest.

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