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3D Printing Geometric Scaffold Design Variation of Injectable Bone Substitutes (IBS) Paste

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Abstract. 3D printing technology application in tissue engineering could be provided by designing geometrical scaffold architecture which also functionates as drug delivery. For drug delivery scaffold on bone tuberculosis, the cell pore of the geometric design was filled with *Injectable Bone Substitutes* (IBS) which had streptomycin as anti-tuberculosis. In this study, scaffolds were synthesized in three cells geometric filled by *Injectable Bone Substitutes* (IBS), *Hexahedron, Truccated Hexahedron,* and *Rhombicuboctahedron*, which had 2.5 mm x 2.5 mm x 2.5 mm size dimension and 0.8 mm strut. The final design was printed in 3D with *polylactic acid* (PLA) filament using the FDM process (*Fused Deposition Modelling*). The composition of IBS paste was a mixture of hydroxyapatite (HA) and gelatine (GEL) 20% w/v with a ratio of 60:40, streptomycin 10 wt% and *hydroxypropyl methylcellulose* (HPMC) 4% w/v. It was then characterized using Fourier-transform infrared spectroscopy (*FTIR*). Scaffold–paste characterization was included pore size test of 3D printing result before and after injected using S*canning Electron Microscope* SEM, porosity test, and compressive strength test. The result showed that the pore of scaffold design was 1379 µm and after injected with IBS paste, the pore leaving 231.04 µm of size. The scaffold with IBS paste porosity test showed ranges between 40,78-70,04% while the compressive strength of before and after injected ranges between 1,110-634 MPa and 2,217-6,971 MPa respectively. From the test results, the scaffold 3D printing with IBS paste in this study had suitable physical characteristics to be applicated on cancellous bones which were infected by tuberculosis.

INTRODUCTION

Three dimensional (3D) *printing*, also known as *Additive Layer Manufacturing*, is a process to create objects in 3 dimensional or any other form from the digital model. 3D printing can be used widely to produce an object based on its complex geometries and interfaces design [1]. Scaffold design in tissue engineering is one of the applications of 3D printing in medicine to repair organ or tissue injury. In 2015, Jian An reported an experiment of 3D tissue and geometric scaffold design with 20 different polyhedral forms in unit cells [2]. Tan also fabricated the scaffold 3D printing in 2014, using alginate-based to construct tissue engineering [3]. In 2015, Chia upgraded the resolution of 3D printing using biomaterial polymer while Itoh Manabu was analyzing scaffold-free tubular tissue which created using Bio–3D Printer and proving cells remodelling and endothelialization when implanted in rat aortae [4–5]. An ideal 3D scaffold should have interconnected pores structure that can ease the cells proliferation, migration, and infiltration [6]. In 2016, Singh observed the function of scaffold on helping skin tissue regeneration and healing the injured areas [7].

Scaffold holds an essential role on keeping the shape of bone growth and also can be used as drug delivery by adding *Injectable Bone Substitutes* (IBS) paste and providing cells remodelling and regeneration materials on pores [8]. In 2014, Maulida synthesized IBS paste based on hydroxyapatite (HA), gelatine, and streptomycin for spinal tuberculosis. 3D printing innovation on scaffold printing and addition of IBS into scaffold pores facilitated the tuberculosis drug delivery process right on target and accelerated bone regeneration.

Fused Deposition Modelling (FDM) is one of a 3D printing technique by melting and extruding thermoplastic filament through the nozzle of layer by layer scaffold which is designed on printing area. At first, the scaffold was designed using *Computer-Aided Design* (CAD) software and then converted to .STL format with *Polylactic Acid* (PLA) filament. PLA is one of a plastic polymer which is made of biodegradable materials, such as corn, tapioca flour, or processed sugar cane. PLA filament was used on this scaffold printing study because its characteristics qualified the requirements of a scaffold; biodegradable, biocompatible, non–carcinogenic to local tissue, and also not impaired the tissue healing process. PLA is also environmentally friendly since it can be degraded naturally by light and bacteria hydrolysis so that it will be degradable in soil, recyclable, and renewable [9].

This study will discuss scaffold printing with polyhedral geometric design variation, *Hexahedron, Truccated Hexahedron, and Rhombicuboctahedron,* with 2.5 mm length of sides of unit cells and 0.8 mm strut using 3D printing method which then was added by IBS paste. The characterization included FTIR analysis to identify the functional groups of the IBS paste sample, and scaffold porosity test was calculated using Equation 1 as follows :

$$\text{Porosity} = \frac{\text{Ww}-W_d}{\text{Ww}-W_l} x100\% \quad (1)$$

Where :
W_d = Initial *Scaffold* weight (g)
W_l = Wet *Scaffold* weight (g)
W_w = Dry *Scaffold* weight (g).

Morphology test was carried out using the S*canning Electron Microscope* (SEM) to identify the pore size of the scaffold sample before and after injected with IBS paste. Equation 2 was used to obtain the scaffold compressive strength as follows:

$$\sigma = \frac{F}{A} \quad (2)$$

Where σ was compressive strength (MPa), F (N) was the given force, while A (cm^2) was the surface area of the *scaffold* sample.
1 MPa= 1 N/mm^2 = 10 kg/cm^2.

EXPERIMENTAL METHOD

Nano hydroxyapatite used in this study was obtained from Badan Tenaga Nuklir Nasional (BATAN) Jakarta, Indonesia originated from the fish scales. The gelatin was derived from cow skin purchased from 150 bloom Rousselot (Guangdong, China). The streptomycin sulfate (powder for injection) was obtained from PT. Meiji Indonesia. The hydroxypropyl methylcellulose (HPMC) was obtained from Sigma Aldrich H7509.

The *injectable bone substitute* (IBS) was synthesized by dissolving 20 w/v% gelatin (GEL) in deionized water at a temperature of 40ºC for one hour. The hydroxyapatite (HA) powder was added to that solution with the ration of HA and GEL 60:40, 10wt% Streptomycin was added in the mixture. Meanwhile, HPMC 4% w/v was dissolved in distilled water at 90ºC then added to the solution of gelatin, hydroxyapatite and streptomycin at 40°C and stirred for six hours to produce a white IBS.

The tools used were freezer and lyophilizer, 10 cc syringe, viscotester VT-04F RION, pH meter Benchtop OAKTON, and SEM FEI Inspect S50 Japan, while on designing the scaffold geometric 62-bit specification and 2 Gb RAM of Asus Laptop and AutoCAD 2012 program were used. The unit cells of the scaffold were first designed using "*polyline*" command and then directed to the *Drawing Area* to create a scaffold outline in AutoCAD. The finished scaffold was then reproduced using "*copy*" command on the *Ribbon Bar*, saved in .STL format, and printed in 3D using FDM technique.

The results of the unit cells scaffold design and 3D printing were then injected with IBS paste which made of hydroxyapatite, gelatine, HPMC, and streptomycin on the pore by using a syringe. The samples were then left for 24 hours to dry in room temperature.

RESULTS AND DISCUSSIONS

Synthesized Result

In this study, the scaffold pores variation were designed in *Hexadron, Truccated Hexahedron,* dan *Rhombicuboctahedron.* Each unit cells were designed in 2500 µm size of length and strut of 800 µm as many as 4 unit cells per 4 cells in unit pores using the AutoCAD. The designs were saved in .STL format and printed in 3D using PLA filament on FDM technique. The unit cells design, scaffold design, and 3D printing results were shown in Figure 1. The print results of scaffold samples were then injected with IBS paste based on hydroxyapatite (HA), gelatine, HPMC and streptomycin using a syringe and then left for 24 hours in room temperature. The final results of injected scaffolds were shown in Figure 2.

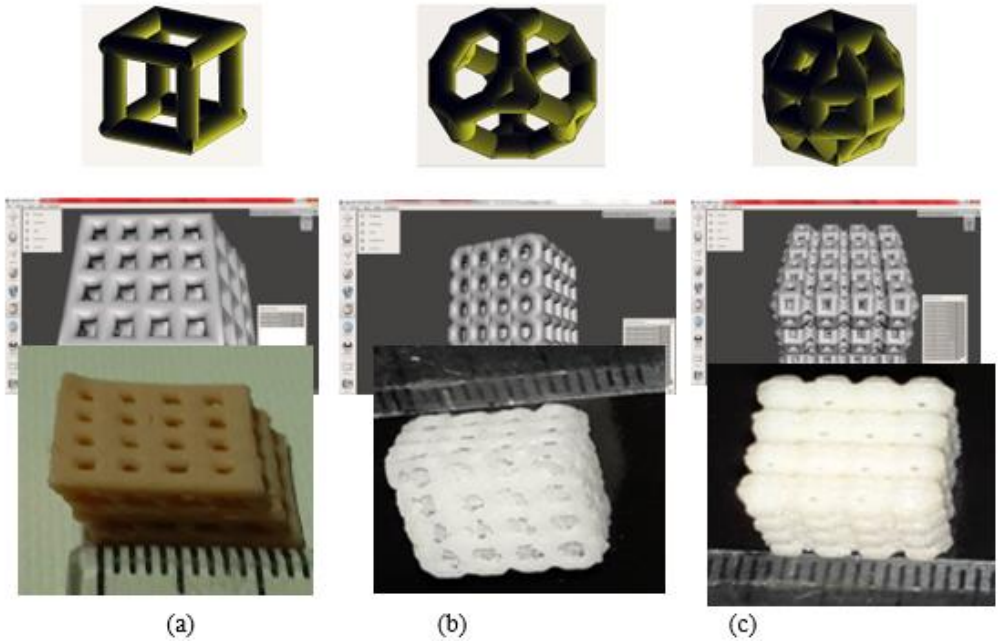

FIGURE 1. The unit cells design, scaffolds design, and 3D printings results :(a) *Hexadron, (b) Truccated Hexahedron dan (c) Rhombicuboctahedron*

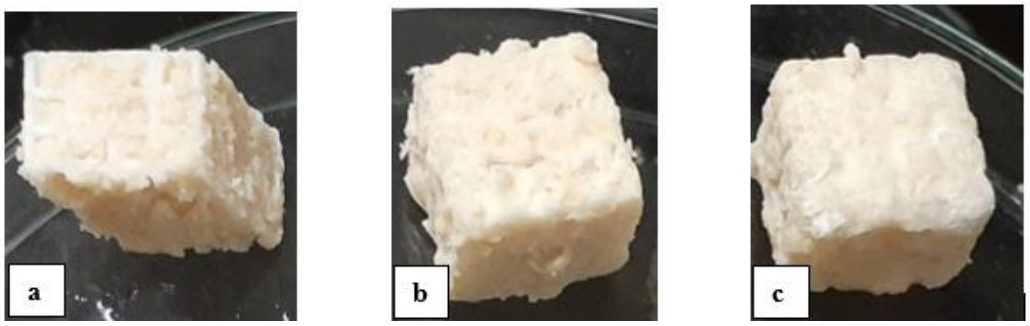

FIGURE 2. The final results of injected scaffolds: (a) *Hexadron,* (b) *Truccated Hexahedron*, (c) *Rhombicuboctahedron*

Characterization Result

The results of the scaffold with IBS paste were then characterized and analyzed using FTIR analysis, porosity test, SEM analysis, and compressive strength test.

Fourier-Transform Infrared Spectroscopy (FTIR) Analysis Result.

Infrared spectroscopy was carried out to identify the functional groups of the used synthesized material. The wavelength used on this FTIR analysis ranges between 4000-400 cm^{-1} for each IBS paste ingredients; hydroxyapatite (HA), gelatine, HPMC, streptomycin, and dried IBS paste sample using the *freeze-drying* method. Figure 3 presented the obtained spectrum.

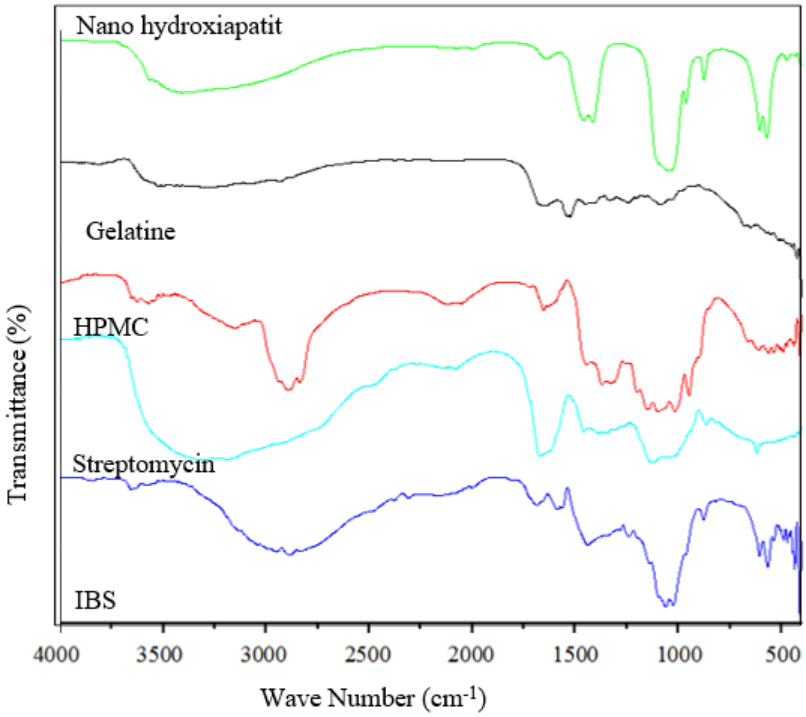

FIGURE 3. FTIR spectrum of IBS paste sample and its ingredients (hydroxyapatite, gelatine, HPMC, and streptomycin).

FTIR spectrum showed that the specific functional groups of dried IBS paste by the *freeze-drying* method could be identified as presented in Table 1.

TABLE 1.. Specific Functional Groups of IBS Paste

Identified Functional Groups	Wave Number (cm^{-1})
Stretching OH vibration	3467.35
Stretching C-CH_3 vibration of HPMC	2929.47
Carbonil (C=O) of gelatine	1648.49
C-NH bending of streptomycin from Amina functional groups	1578.22

Porosity Test Result

Porosity is one of an essential parameter of bone tissue requirement since pores can provide sufficient space and nutrient diffusion for cells to grow. The percentage of porosity (%porosity) on scaffold design and 3D printing paste result of *Hexadron, Truccated Hexahedron dan Rhombicuboctahedron*, were 62.31± 0.002%; 70.04 ± 0.005%; and 40;78 ± 0;002% respectively. An ideal scaffold for bone cancellous should have 70% of %porosity [11]. The biggest %porosity was shown by *Truccated Hexahedron* for 70.04 ± 0.005%, which was ideal for bone cancellous.

Scanning Electron Microscopy (SEM) Analysis Result

SEM analysis of scaffold characterization was carried out to obtain the diameter and morphology of the sample surface before and after injected with IBS paste. This study used SEM magnification of 50x to *Truncated Hexahedron* scaffold pore design, as this sample had the highest %porosity. Figure 4a and 4b presented the result of SEM analysis. The strut structure of layer by layer pores barrier after PLA filament cumulation, and 1379 µm pore size showed in Figure 4a. Meanwhile, Figure 4b presented the pore size of 231.04 µm after injected with IBS paste. Smaller pore size showed that IBS paste was able to fill the scaffold pore and harden or setting. Based on the SEM analysis result, the *Truncated Hexahedron* of scaffold pore design qualified the pore bones size, range between 100 – 300 µm, so it was suitable for *osteoblast* growth and helped the formation of bone tissue [12].

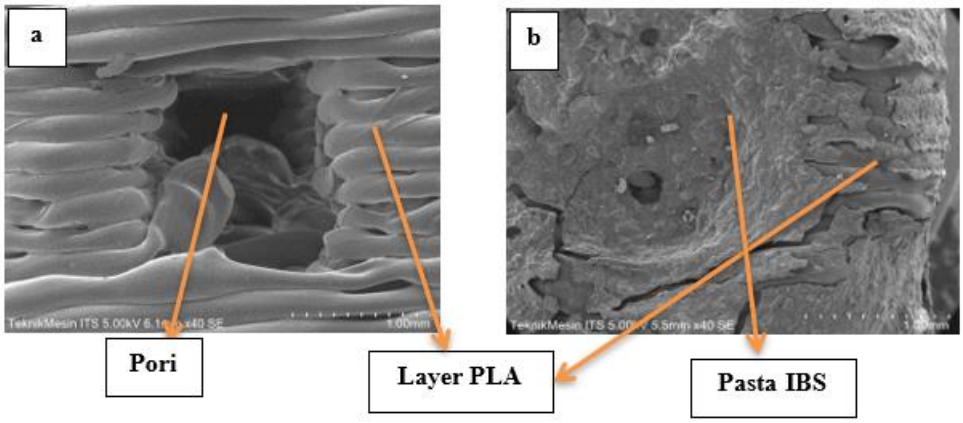

Figure 4. SEM analysis result of *Truccated Hexahedron* scaffold pore design*:* (a) before injected with IBS paste and (b) after injected with IBS paste.

TABLE 1. compressive strength test result of PLA scaffold before and *after injected with IBS paste*

Unit Cells of Pore Design	*Compressive Strength Before Injected with IBS Paste* (Mpa)	*Compressive Strength After Injected with IBS Paste* (Mpa)
A. Hexadron	1.11 ± 0.089	2.22 ± 0.185
B. Truccated Hexahedron	2.99 ± 0.240	5.42 ± 0.452
C. Rhombicuboctahedron	6.43 ± 0.516	6.97 ± 0.581

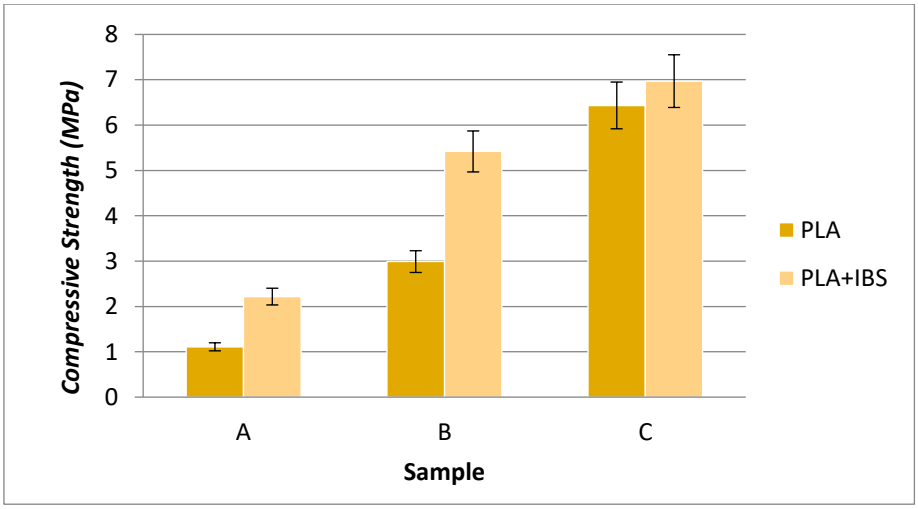

Figure 5. A compressive strength test result of the scaffold.

The addition of IBS paste into the scaffold increased the compressive strength of samples as presented in Figure 5. The lowest increment of compressive strength was on *Rhombicuboctahedron* unit cell scaffold design since it had a smaller pore than *Truccated Hexahedron* so the amount of injected IBS paste also lesser. The compressive strength of cancellous bone substitute was between 2-12 MPa and between 1.5-7.8 MPa for vertebrae [13]. From the compressive strength test result, all of the variations of scaffold design qualified for cancellous vertebrae bone scaffold.

CONCLUSION

3. The 3D printing result of geometric design scaffold indicated layer by layer of PLA as the scaffold partition. The pore of *Truccated Hexahedron* scaffold design was 1379 µm of size and filled by IBS paste after injected. FTIR analysis result on IBS paste showed that no new functional groups formed.
4. The porosities of scaffolds with IBS paste were between 40,78–70,04 %, and *Truccated Hexahedron* had the biggest pore design. From SEM analysis, *Truccated Hexahedron* left a pore size for 231.04 µm after injected with IBS paste.

The compressive strength of this scaffold variation was 1.110-6.434 MPa and increased for 2.217-6.971 MPa after injected with IBS paste.

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