Physical Characterization of Injectable Bone Substitute Associated-3D Printed Bone Scaffold for Spinal Tuberculosis

I F Wardhani¹, R M R Samudra², Katherine³, D Hikmawati⁴, Aminatun⁵
¹,²,³ Biomedical Engineering Study Program, Universitas Airlangga, Indonesia.
⁴,⁵ Physics Department, Universitas Airlangga, Indonesia.
(usi.ifw@gmail.com, rofimega400@gmail.com, katherinehouw@yahoo.co.id)
Co-author : dyah.hikmawati@yahoo.co.id

Abstract. There are two important factors to overcome spinal tuberculosis, i.e. killing and preventing the Mycobacterium tuberculosis to spread, also replacing and reconstructing the damaged bone. This study focused on an innovation to overcome the spinal tuberculosis problem by combining the IBS and 3D printed bone scaffold. This innovation could provide a local drug delivery system along with tissue regeneration and bone reconstruction. In this study, 3D printed bone scaffold which have been injected with IBS were tested its physical characterizations. The bone scaffold was fabricated from Polylactide acid (PLA) filament using FDM method through an ordinary commercial 3D printer. The IBS was synthesized from nanohydroxyapatite, gelatin, streptomycin, and HPMC. The physical characterization performed in this study were porosity test functional group test, and degradation test. From those characterizations, it could be concluded that the IBS associated-3D printed bone scaffold is a potential alternative to overcome spinal tuberculosis.

1. Introduction
It was reported that Indonesia still be the 3rd country worldwide with the most tuberculosis (TB) sufferers in 2017. This disease was one of 10 main causes of the death among the people in all over the world [1]. In particular cases of TB, when it got more serious, the bacteria could also infect the other organs, including the musculoskeletal organs. The plenty vascularization to the spinal bone causes the bacteria, which previously had been lodged in the lungs, to reactivate in this spot. This case is named as spinal tuberculosis, which was reported to reach up to 50% of the musculoskeletal tuberculosis case in total [2].
Spinal tuberculosis could cause the disturbance of nerve system and permanent deformation of the spine. The medical practitioners offers a conventional treatment through the drugs. This kind of treatment needs a high level dose of medicine, yet it also needs a long time treatment to be able reach the spot of bacterial infection [2]. While the bacteria grew in the spine, it will cause the bone destruction which has to be corrected by the surgical treatment. The surgical treatment was necessarily done to remove the infected bone tissue, then replace it with the bone graft [3].
Scaffold was one kind of bone graft which offers to help the new bone cell to grow as well as to reconstruct the geometry of the spine. The scaffold must meet some characteristics, i.e. biocompatible, an appropriate pore interconnectivity, also suitable pore size and shape [4]. To provide those characteristics, a new technology, which has been developed in the era of Industrial Revolution 4.0,
known as additive manufacturing, offers an efficient and low cost way to fabricate the bone scaffold by using 3D printing technology [5]. The fabrication could simply be done, started by designing the bone scaffold using CAD (Computer Aided Design) application. This application allowed us to design a custom bone scaffold based on the patient’s case need.

Injectable bone substitute (IBS) paste which was synthesized by loaded the antituberculosis drug, i.e. streptomycin, had been proven to be an alternative to overcome the spinal tuberculosis [6]. This research was aimed to synthesize the 3D printed bone scaffold, then combine it with IBS paste to be a more effective alternative to be able overcoming spinal tuberculosis. This idea was due to the need of bone geometry reconstruction and urge to kill the bacteria so that it could not spread to the wider area.

2. Materials and Method

2.1. Materials

The materials used in this study included Polylactid Acid (PLA) filaments to make bone scaffold. While, the main ingredient for making IBS paste, including nanohidroksiapatit powder, gelatin, Hydroxypropyl Methylcellulose (HPMC), and streptomycin sulfate (powder for injection) packaging 1 gram vial. In addition, materials that are useful as solvents, namely aquades, are also needed.

2.2. Bone Scaffold Manufacturing

The manufacturing of bone scaffold was begun with designing process using Autodesk AutoCAD 2019-Student Version. The scaffold was built by ordering a single fibre to one another. The space between 2 fibres would be the pore size of the scaffold. The fibre was designed using a 2-dimensional line with 14 mm length and a circle with 1.2 mm diameter. The circle was then swept using “sweep” command along the line as shown in the Figure 1.

![Figure 1](image.png)

Figure 1. Top view of (a) The making of single fibre which consisted of a line and circle; (b) The circle that had been swept along the line; (c) The fibre in conceptual view

The single fibre was then copied and ordered side by side with the 1.2 mm space between one another to make the 1st layer. The 2nd layer was made from the 90° rotation of the 1st layer, as could be seen in Figure 2. Furthermore, the 3rd and 4th layer were the copying result of the 1st and 2nd layer. The process was then repeated until the 14th layer had been made. The final design which had been made could be seen in Figure 3.
Figure 2. The design of 1st and 2nd layer for bone scaffold manufacturing

Figure 3. The final design of bone scaffold

Extension from the AutoCAD 2019 application in the form of .dwg was then converted to the .stl form via the Cura from Ultimaker application by looking at the slicing parameters of the design. This stereolithography (.stl) extension could later be read by a 3D printer machine, so the design can be printed. Slicing parameters themselves were a layer-by-layer display that will be printed using a 3D printing machine. This was important to know because from here we could determine whether the design can be printed by a 3D printer machine. Figure 4 would show the slicing parameters of the scaffold design. The filament used is PLA with a diameter of 1.75 mm. To make a good print accuracy, a nozzle is used with hole diameter of 0.2 mm.

Figure 4. 3D design slicing parameter of bone scaffold
2.3. **IBS Paste Synthesis**

Based on research conducted by Maulida, et al (2015) IBS paste was made with the main ingredients nano hydroxyapatite, gelatin, HPMC, and streptomycin. At first 20% w / v gelatin was dissolved in distilled water at 40°C for 1 hour. In the same time, HPMC 4% w / v was dissolved in distilled water at a temperature of 90°C for 1 hour then left to stand until the temperature of the solution became 40°C. In the gelatin solution that had been made, hydroxyapatite nano powder was added according to the ratio of the composition of HA: gelatin 65:35 (w / w), this mixture was then stirred for 1 hour. After both were homogeneous, the next step was adding 10% streptomycin of the total mass, then stir until homogeneous. Furthermore, the HPMC solution was mixed into HA-gelatin-streptomycin at a mixing temperature of 40°C with stirring for 6 hours.

2.4. **Sample Characterization**

Porosity test was conducted to know the percentage of pores contained in the scaffold. Scaffold porosity can be calculated using Equation 1 [7]. The density value of the material used, namely PLA, is 1.24 g / cm³ [8]. Where \( V_{\text{scaffold}} \) was volume of scaffold and \( w_{\text{scaffold}} \) was mass of scaffold.

\[
\text{porositas} \, (\%) = \left( \frac{V_{\text{scaffold}} - w_{\text{scaffold}}}{V_{\text{scaffold}}} \right) \times 100\% \quad (1)
\]

Then, the IBS associated 3D printed bone scaffold were characterized its chemical identity using FTIR (Thermo Scientific Nicolet iS10). The method used was attenuated total reflectance (ATR). The 3D printed scaffold was tested before and after the IBS injection. Both samples were placed in sample holder, then recorded.

The in vitro degradation test was conducted using Phosphate Buffer Saline (PBS) solution which represented the body liquid. The 3D printed scaffold was soaked in PBS solution. The scaffold mass and PBS pH value were then evaluated every 6 days in 18 days.

3. **Result**

3.1. **IBS Associated 3D Printed Bone Scaffold**

The 3D printing for bone scaffold fabrication was shown satisfactory result as could be seen in Figure 5. It could be seen that macroscopically, the pore and its interconnectivity were very well printed. Using image J application, the scaffold pore size measured and resulted 1172.614 µm in average. From that measurement, it could be known that the capability of the 3D printing to print the scaffold compared to the design was 97.718%. While the porosity of the bone scaffold was measured and resulted 65.329 ± 0.603 % value of porosity. The pores of the bone scaffold were then filled by the IBS paste which had been able to be made and loaded with antituberculosis drug. The IBS associated 3D printed bone scaffold could be seen in Figure 6.

![Figure 5. 3D printed bone scaffold before IBS injection](image1)
![Figure 6. 3D printed bone scaffold after IBS injection](image2)
3.2. FTIR Test

The FTIR test was conducted to know the functional group of the bone scaffold before and after the IBS injection. In the Figure 7 provided the FTIR test result of both samples. The chemical identity of scaffold showed typical functional group spectrum of PLA. Stretching C-H spectra were found at wavenumber 2999.93 and 2944.39 cm\(^{-1}\), C=O bonding spectrum was at 1744.57 cm\(^{-1}\), and C-O was found at range 1179.69 until 1041.74 cm\(^{-1}\).

What made different between scaffold before and after the IBS injection were shown by existence of O-H functional group at 3266.24 cm\(^{-1}\), stretching N-H at 1636.77 cm\(^{-1}\), and PO\(_4\)^3~ bonding at 599.33 cm\(^{-1}\). Those functional groups were the specific IBS paste ingredients’ spectra.

![Figure 7. The FTIR test results of bone scaffold before and after the IBS injection](image)

3.3. In Vitro Degradation Test

The degradation test was conducted to ensure that scaffold based on PLA was a biodegradable material. After 18 days, the mass of scaffold had not changed significantly, as could be seen in Figure 8. Otherwise, there was pH value decreasing over time, as shown in Figure 9.

![Figure 8. The changing of bone scaffold in 18 days](image)
Figure 9. The PBS solution’s pH value decreasing in 18 days of scaffold soaking

4. Discussion

It had been proven that 3D printing technology could be used to fabricate bone scaffold with 97.718% accuracy compared to its design. The scaffold, which was fabricated in this study, had porosity above 50% that meant it was like any common scaffold which could be found in the market [5]. The fabrication of IBS associated-scaffold was successfully done, indicated by its chemical identity. From the FTIR test, it was known that the sample had specific functional group spectra from its ingredients. One of important indicators of scaffold is the biodegradable character. Scaffold was implanted to be an extracellular matrix that could help the new osteoblast growth [4]. When bone has already grown appropriately, the bone scaffold itself has to be degraded by human body fluid. It had been proven in this study that bone scaffold based on PLA was a biodegradable material, which could be seen on the data explained in section 3.3. Although, there had not been mass decreasing in 18 days, the pH value decreasing could be shown as a proof that the scaffold was biodegradable. The decreasing pH value indicated that there was monomerization process during the scaffold soaking. PLA would be degraded by PBS and produced lactic acid.

The degradation profile of IBS paste itself was not studied more deeply in this study. From the results of the study of Maulida, et al (2015) showed that the dried IBS paste could be degraded within 48 hours in a Simulated Body Fluid (SBF) solution. This can be a reference that IBS paste would be degraded first and released the drug to kill bacteria before the PLA scaffold degradation process happened. This ability of killing bacteria had been proven by Maulida, et al (2015) in their research. It had been tested by using Saccharomyces aureus (SA) and resulted very sensitive results on the SA inhibition zone [6]. The degradation of IBS paste would also decompose the minerals contained in its constituent ingredients, especially the minerals contained in nano HA. These minerals would help the process of bone regeneration through the process of osteoconductivity [9]. After this IBS paste degradation process, it was hoped that the bacteria that caused spinal tuberculosis had been resolved and new bone cells (osteoblasts) could begin to grow. The next healing mechanism is to make bone scaffold as an extracellular matrix for osteoblasts to attach to and develop into bone tissue as before. Until finally the scaffold will degrade by itself when new bone tissue has formed to replace bone damaged by bacteria.

5. Conclusion

In conclusion, 3D printing technology could be potentially utilized in medical area, especially to overcome spinal tuberculosis. The IBS associated-3D printed bone scaffold is a potential alternative regarding to this case. For future study, there is still a need to develop this research to know whether the scaffold could really control the drug release by its pore size and design.
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References
[1] World Health Organization 2018 Global Tuberculosis Report Executive Summary (Geneva: WHO)
[2] Shaikh N S and Sawakar S P 2017 iMedPub J., 4 1
[3] Do Brito J S, Tirado A and Pedro F 2014 The Iowa Orthopaedic J., 34 129
[4] Dorati R, DeTrizio A, Modena T, Conti B, and Benazzo F 2017 Pharmaceuticals, 10 96
[5] Gregor A et al 2017 J. of Biol Eng., 11 11
[6] Maulida H N, Hikmawati D, and Budiatin A S 2015 J. of Spine, 4 266
[7] Tanaka Y et al 2010 Biomaterials, 31 4506
[8] Rodrigues N et al 2016 Procedia CIRP, 49 33
[9] Kattimani V S, Kondaka S, and Lingamaneni K P 2016 Bone Regeneration. Bone and Tissue Regeneration Insights, 7 9