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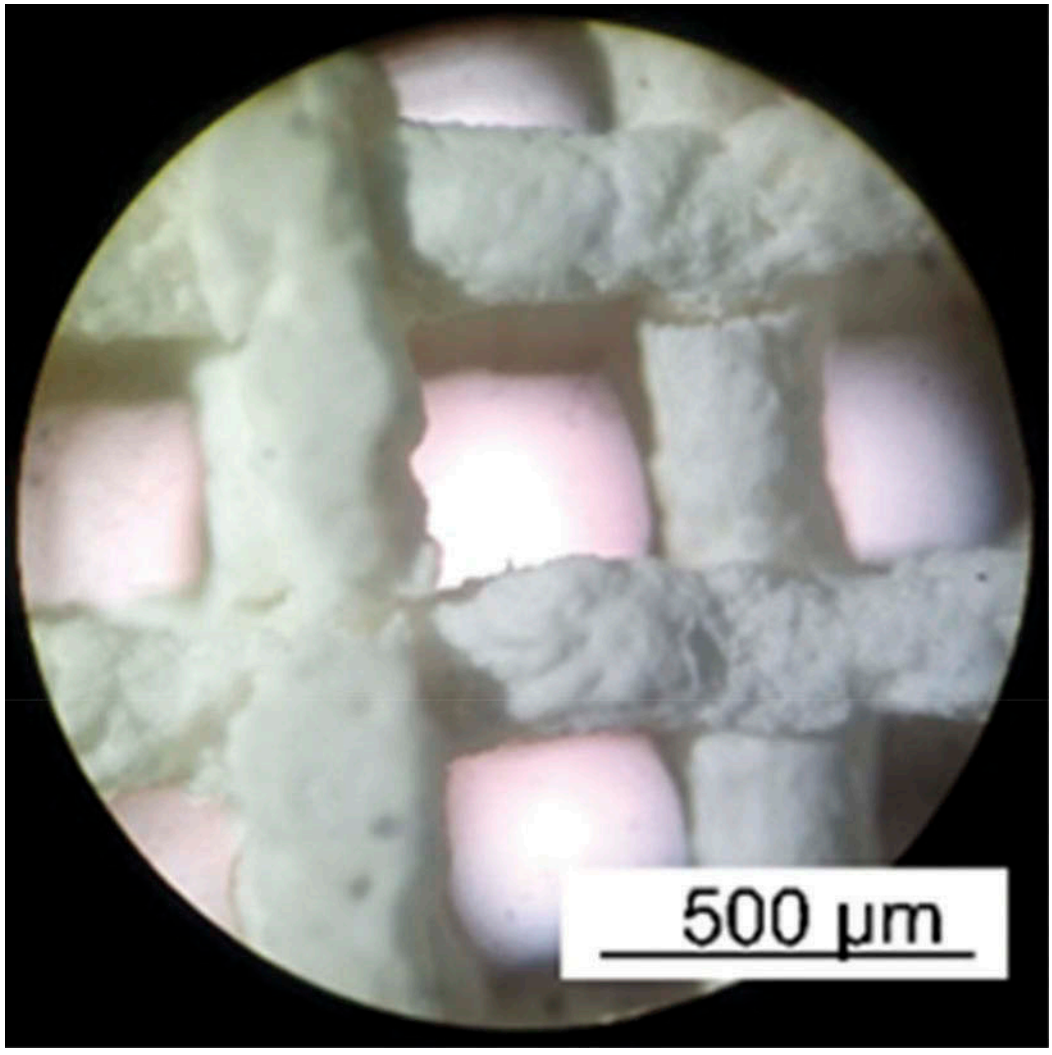

BIOMEDICAL ENGINEERING | RESEARCH ARTICLE

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The effect of extruder pressure of 3D bioprinter on hardness and compressive of scaffold bovine hydroxyapatite

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Abstract: The aims of the study are to find out bio-printer extruder pressure effect toward the hardness and compressive strength of bovine hydroxyapatite (BHA). BHA and Glycerin are mixed with a 1:1 ratio. After 24 h bioink will be formed and can be used as a material to print scaffold. The scaffold is printed with a pressure variation of 100, 150, 200 and 250 KPa. After the printing process is done, the scaffold is kept on heat bed for 10 min with 100°C temperature. The hardness test result in this study has the highest value of 24.23 ± 1.22 HVN and 2.03 ± 0.12 HVN for its lowest. The compressive strength test has the highest value of 5.71 ± 0.42 MPa with the lowest value of 3.22 ± 0.13 MPa. The observation of BHA scaffold pores using a microscope shows that the pores have an average size of 100–200 µm.

Subjects: Materials Science; Biomaterials; Biomedical Engineering

Keywords: Bovine hydroxyapatite (BHA); glycerin; scaffold; 3D printing

1. Introduction

Indonesia is a country with a high number of fractures. Broken human bones can be cured by the bone graft process. Most of the materials used in the bone graft process are autographs. Autographs have weaknesses, increase the risk of infection and blood loss, increase anesthesia time and limited development.

Joko Triyono

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Joko Triyono received his BE degree from Sepuluh Nopember Institut of Technology (ITS) Surabaya in year 1993 and Master of Technology at the same University in year 2003. He passed Ph.D degree from Gadjah Mada University (UGM) Yogyakarta in year 2015 in Biomedical Engineering Study Program. Since 1997, he has been working at Mechanical Department Sebelas Maret University (UNS) Surakarta, Indonesia. He has published several papers in International and journal. Until now, his research in the field of biomedical engineering included the development of material in the orthopedic field such as the development of bone graft material from bovine bone material and eggshell, the use of shellac as a filler for biocomposites material and the development titanium implant material based on CT Scan data.

PUBLIC INTEREST STATEMENT

Bone graft is an implant material used in the orthopedics field as a bone filler material in cases of bone fractures. The function of bone graft is as a bridge of growth so that new bones can grow and re-union. Some materials are used for this material, for example, eggshells, gypsum, coral and bovine bones. In this activity, it was reported the results of research on the development of bone graft from biocomposites of bovine hydroxyapatite and glycerin. The printing process of bone graft is done by 3D printing technology.

It was reported from this study that bone graft scaffold can be produced by 3D printing technology. The produced bone graft size is 10 × 10 × 10 mm with a pore size of 100–200 µm and wall thickness of 350–500 µm. Based on the hardness, compressive strength and porosity that have been tested, this material has similarities to the products on the market.

Weakness of the autograph opens the opportunity to develop synthetic bone grafts. One function of synthetic bone graft is as a scaffold. Hydroxyapatite is one of the most widely used ingredients for making synthetic bone grafts. The main characteristic of hydroxyapatite is that it has biocompatibility, bone bonds, and can grow and develop together with the original bone or good bone regeneration.

According to Meskinfam, Sadjadi, Jazdarreh, and Dan Zare (2011), there is a lot of research that uses bovine bones to be used as hydroxyapatite material as one of the bioceramic products. A research was conducted by O'Kelly, Tancred, McCormack, and Dan Carr (1996) shows that bovine bone has a structure consisting of HA and about 8–10% tricalcium phosphate so that it is suitable to be used as a scaffold. According to Krishnamurithy et al. (2014), hydroxyapatite scaffold of bovine bones provides a good physiological environment for cell integration and development.

Shor, Guceri, Wen, Gandhi, and Dan Sun (2007) state that designed scaffolds as bone grafts must have a high level of porosity and interconnectivity to support cell integration and development. Scaffold must have structural integrity that matches the nature of the original network. Pore size in the scaffold must also be suitable for nutrient transport. Most existing scaffold fabrication methods such as solvent casting, fiber bonding, phase separation, gas-induced foaming and salt leaching are limited to producing scaffolds with simple geometry. This traditional scaffold fabrication method produces an irregular and highly variable inner structure.

According to Malda et al. (2013), an alternative method that can be used to fabricate scaffolds is to modify the extruder system in 3D printers. Scaffold material is generally inserted into the injection and removed using a piston system. The piston type extruder produces a continuous flow of material. Besides that the fabrication speed using piston type extruder is quite high and is able to produce scaffold shapes that are in accordance with anatomy. The material on the piston type extruder can also flow well. With these advantages, the piston type extruder allows for the fabrication of relevant size scaffolds within a realistic time limit so that this technology is very promising.

2. Method

2.1. Making 3D bio-printer

This study consists of two stages, namely 3D Bio-printer design made from acrylic glass and the process of printing bovine hydroxyapatite (BHA) scaffold specimens. The research process starts with reference studies related to the use of 3D printing methods in hydroxyapatite scaffold fabrication. The next process is to design a 3D Bio-printer by using four stepper motors to drive the x, y, z and extruder axes. The controller is equipped with three end-stop to keep the machine from moving over a predetermined distance.

The dimension of the 3D printer was 300 × 310 × 300 mm, has a working dimension of 10 × 10 × 10 cm based on acrylic and stainless steel materials construction Ø8 mm. The motion mechanism used in this operation was a pulley and with a stepper motor belt (Figure 1).

2.2. Printing of scaffold BHA

After designing and building 3D Bioprinter, the printing process of BHA scaffold specimens was carried out with extruder pressure independent variables, namely 100, 150, 200 and 250 KPa, and the control variable was heatbed temperature of 100°C. The ratio of hydroxyapatite and glycerin used is 1:1. The printing stage for BHA scaffold specimens starts with the creation of a scaffold model with Solidworks and stored in the STL format. Then, STL is converted to G-code using the Repetier-Host software and inputted into the SD Card. SD Card was inserted into LCD Display which has been equipped with SD Card Reader. The process then regulates the temperature of the heat bed and heats the heat bed to the desired temperature of 100°C. Then, choose the G-code that will be printed and wait until the BHA scaffold specimen printing process is completed.

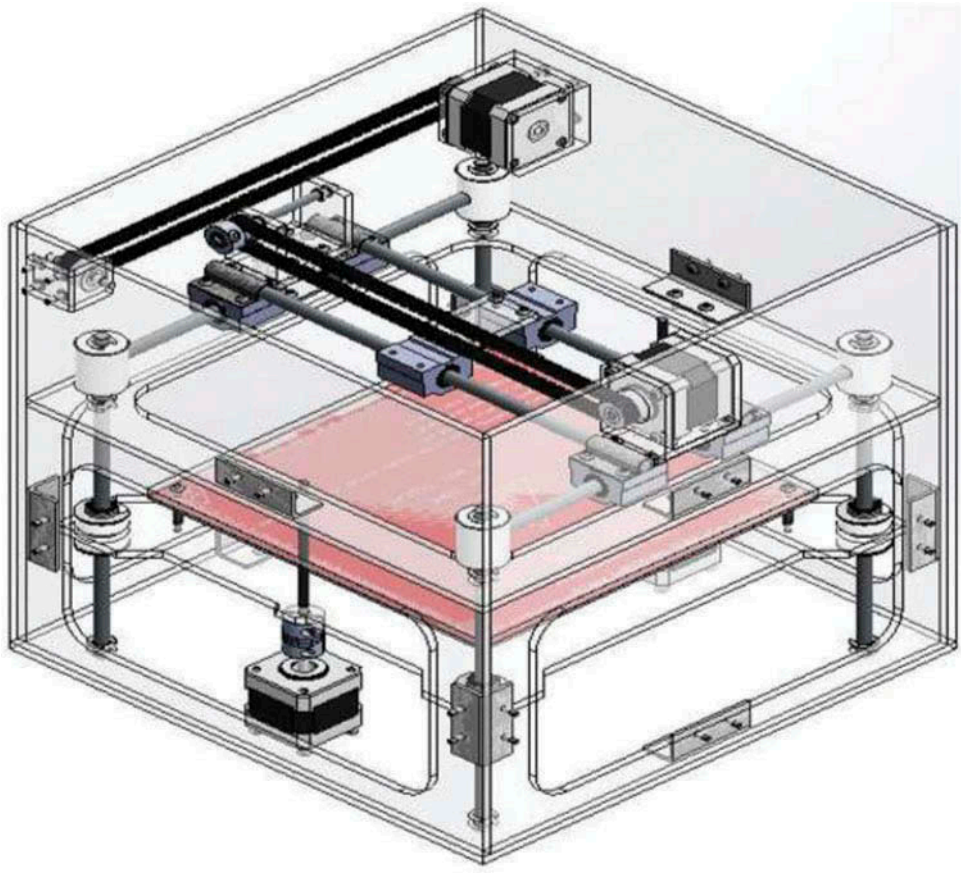


Figure 1. Design of scaffold 3D printer (Triyono, Pratama, Sukanto, Nugroho, & Wijayanta, 2017).

3. Results and discussions

3.1. Effect of 3D bioprinter extruder pressure on BHA scaffold structure

The effect of 3D extruder pressure variations of 100–250 KPa bioprinter shows the difference in BHA scaffold structure. The higher the 3D bioprinter extruder pressure causes Bio-ink to come out too quickly so that it is less able to maintain the BHA scaffold form. On the contrary, the lower the extruder pressure can produce a BHA scaffold form which is maintained in the groove.

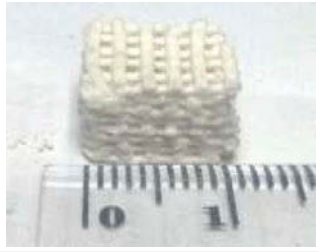
The results of BHA scaffold printing with various variations of 3D bio-printer extruder pressure are shown in Table 1.

The results of 3D printing of BHA-glycerin scaffolds show that it can be deposited by using a 3D printer. From the printouts of bio-ink with a pressure of 100–200 KPa, the average scaffold size is 10 × 10 × 10 mm and produces interconnected pores. The printed 3D scaffold to be hard after being left in the heatbed for 10 min and dried in a microwave.

At 250 KPa, the 3D printer cannot produce 3D scaffolds. This is due to the pressure that Bio-Ink is released too quickly so it overflows. The problem that occurs causes the 3D printer to be unable to maintain the printed form.

Bio-ink used in this study is in the form of a slurry, the slurry properties of bio-ink must be stable and uniform during extrude processing by 3D printers. The level of stability and uniformity of Bio-Ink slurry has been studied by Zhou et al. (2015) by stating that HA slurry (30% vol) was prepared by adding Glycerol (30% wt) in water; then, the mixture was added 2 wt% poly-carboxylic acid to

Table 1. Results of BHA scaffold printing

Extruder pressure (KPa)	*Scaffold* BHA	Structure
100		Pore formed
150		Pore formed
200		Pore formed
250		Any pores are closed

increase uniformity and stability of slurry. After the slurry was prepared, then the slurry was extruded using the micro-syringe extrusion system. After that, the extruded scaffold was heated using a microwave at a temperature of 100°C for 10 min to improve the mechanical properties of the scaffold.

3.2. Result of hardness test

The results of BHA-Glycerin 3D scaffold hardness testing can be seen in Figure 2.

Figure 2, it can be seen a graph of the average of BHA-Glycerin 3D scaffolding linearly decreasing. The greatest hardness occurs in specimens using extruder of 100 KPa. The value then decreases in line with the addition of the extruder. The values were, respectively, of (24.23 ± 1.22 HVN), (12.25 ± 0.80 HVN), (7.50 ± 0.30 HVN) and (2.03 ± 0.12 HVN) at 100, 150, 200 and 250 KPa. Decreasing hardness of BHA-Glycerin scaffolding occurs every increase in 3D bioprinter extruder. Decreasing of BHA-Glycerin biocomposite hardness is due to the increasing velocity of the hydroxyapatite material flow. BHA as a filler material will continue the load distributed by Glycerin as a matrix. The decreasing in violence that occurred from a pressure of 100 –250 KPa was caused by the uneven distribution of the load received by the BHA.

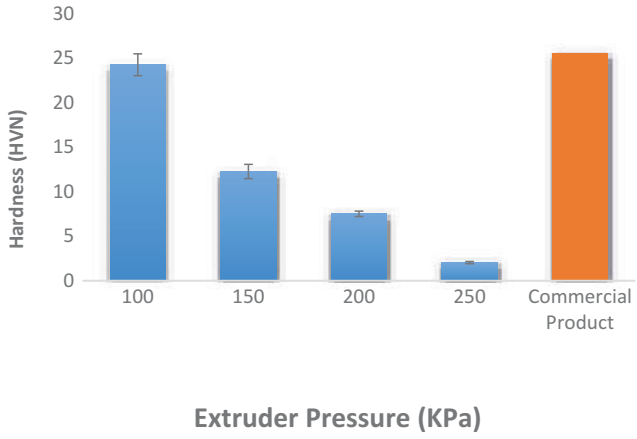


Figure 2. Hardness of BHA scaffold.

Clarizio, and Dan Tatara (2012) stated that the reduction in composite strength was due to the increase in the Glycerol fraction as a filler. The hardness of the composite with the addition of Glycerol decreased with the highest hardness of 79 ± 0.5 HVN and the lowest at 51 ± 5.5 HVN. The highest hardness was on the addition of Glycerin 5% with a value of 0.872 ± 0.081 kg, while the lowest was the material containing the highest Glycerin which was 20% (0.498 ± 0.057 kg) (Laeha, 2015). Based on these values, it can be stated that adding volume of filler will reduce composite hardness.

Based on Clarizio and Laeha's research, it can be stated that the addition of glycerol can reduce the hardness of composite. In this study, the addition of high glycerin concentrations can reduce 3D scaffold hardness. Based on the results of the 3D BHA-glycerine scaffold test from each pressure, it was found that all of hardness value was lower than the commercial BHA scaffold which had a hardness of 25.57 ± 1.07 HVN. Increased glycerin concentration will reduce composite hardness.

3.3. Compression strength

The 3D scaffold compressive strength of BHA-Glycerin was obtained by testing Axial Compressive Strength using Universal Testing Machine (JTM Technology Machine, 0.5 T Capacity) 50 kg load cell and a compressive speed of 5 mm/min. The results of the study were made in the form of graphs shown in Figure 3 BHA-glycerin 3D scaffold compressive strength testing diagram.

It can be seen in Figure 3 that the addition of extruder pressure will reduce the compressive strength of the composite material. The highest value obtained at a pressure of 100 KPa then decreases in line with the addition of extruder pressure. The values of compressive strength were, respectively, 5.71 ± 0.42 MPa, 4.34 ± 0.41 MPa, 3.57 ± 0.13 MPa and the lowest 3.22 ± 0.13 MPa at 100, 150, 200 and 250 KPa.

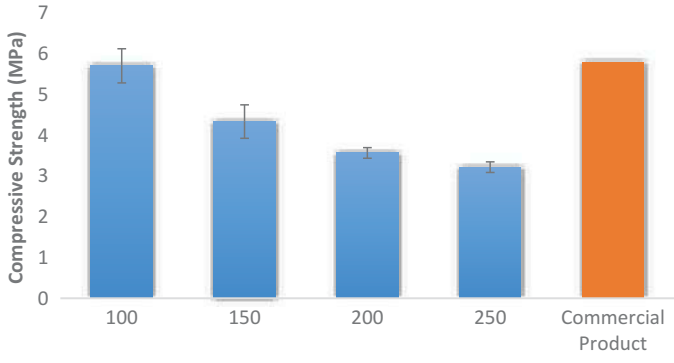


Figure 3. Compressive strength of BHA scaffold.

In this study, a compressive strength test was carried out on commercial HA products. The results of compressive strength were 5.80 ± 0.97 MPa. This value is higher than the 3D results of the experimental material. The decrease in compressive strength in this study was due to the increase in the pressure of the 3D bio-printer extruder. This is due to the increasing speed of hydroxyapatite material flow which makes the 3D scaffold irregular form and makes the material weak. The functions of glycerin as a plasticizer can make biocomposites softer and resistant to impact. Another cause of changes in the BHA-Glycerin compressive strength is because the molecule of glycerin has an interaction force with bovine hydroxyapatite (BHA). If the number of BHA molecules is less than plasticity (glycerin), the molecular interaction force will decrease and cause the composite strength to decrease.

The analysis is in accordance with Wardhani, Rudyardjo, and Dan Supardi (2013) which stated that the addition of glycerol as plasticizer serves to reduce stiffness so that the biocellulose-chitosan is protected from cracking and is more flexible. In this study, the value of tensile strength decreased along with the addition of glycerol. In addition, this happens because the nature of glycerol as a plasticizer is to reduce stiffness to make it more flexible so that the mechanical strength of biocellulose-chitosan also decreases.

3.4. Observation macrophoto of BHA scaffold

Microscope observations were conducted to measure pore size and morphology material as shown in Table 2.

Table 2. Results of BHA scaffold macrophoto

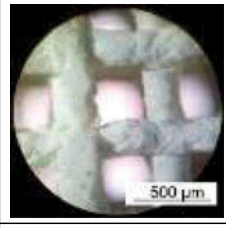
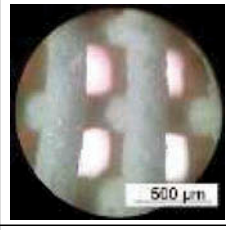
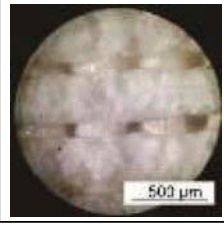
Pressure (kPa)	Macrophoto of BHA *Scaffold*
100	
150	
200	
250	

Table 2 shows macrophoto and porous on BHA scaffold at extruder pressure of 100–250 KPa. The size of porosity is 100–200 mm, and the size of the wall varies from 350 to 500 mm. The pore shape is a square with an interconnecting pore ± 30 × 18 layers, 50% porosity. Each addition of 3D bioprinter pressure causes the layer to become smoother because the BHA granules are less and covered by a layer of glycerin. At the pressure of the extruder 100 and 150 KPa, porous interconnections are formed but at a pressure of 200 and 200 KPa interconnection is not formed. Non-porous interconnection is caused by accumulation of glycerin which closes porosity.

Ozbolat and Dan Hospodiuk (2016) have conducted research on the use of Pluronic as a hydrogel. This study explained that Pluronic requires heated plates to prevent melting of hydrogel and loss of form during the printing process.

The pore size and porosity produced by 3D printers showed success in the deposition of BHA-Glycerin into 3D scaffold. This is confirmed by Huang's (2011) study (Huang et al., 2011) stating that the minimum pore size of 100 µm with 50% porosity must be possessed by the scaffold in order to be able to meet the mechanical properties and new bone growth. In the study also explained that there are four morphologies that are ideal for pores, namely round, elongated or ellipse, column shaped and square or rectangular.

Based on research that has been done that the pressure of the 3D bio-printer extruder determines the success of the 3D printer in depositing Scaffold. The variation of 3D bio-printer extruder pressure of 100–200 KPa has succeeded in making uniform and stable scaffolds. The results of the BHA-Glycerin 3D scaffold hardness test from each pressure obtained a lower hardness value than the commercial BHA scaffold which had a hardness of 25.57 ± 1.07 HVN. The conclusion of this study is that the addition of pressure to the 3D bio-printer extruder decreases the strength of the composite. The 3D printer in this study was able to deposition BHA-Glycerin into 3D scaffold with a pore size of 100 µm, 50% porosity, and square pore morphology.

4. Conclusions

(1) Bone graft scaffold can be made by 3D printing technology from a mixture of BHA and glycerin.

(2) It was obtained interconnected porosity at an extruder pressure of 100 and 150 KPa but at a pressure of 200 and 250 KPa interconnected porosity was not obtained. The size of pore size was 100–200 mm, and the size of the wall varies of 350–500 mm.

(3) The highest hardness of bone graft scaffold is at a pressure of 100 KPa (24.23 ± 1.22 HVN) and the lowest is at a pressure of 250 KPa (2.03 ± 0.12 HVN). Bone graft scaffold hardness decreases by increasing speed in the extruder.

(4) The highest compressive strength of bone graft scaffold is at a pressure of 100 KPa (5.71 ± 0.42 MPa) and the lowest is at a pressure of 250 KPa (3.22 ± 0.13 MPa). Compressive strength of bone graft scaffold decreases by increasing speed in the extruder.

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