Permeability Analysis of Bovine Bone Scaffold in Bone Tissue Engineering

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Abstract

Defects in the mandible cause deformities, functional disturbances and facial esthetics that cause a decrease in a person's quality of life. Autograft is the gold standard because it has osteogenic, osteoinductive, osteoconductive properties and does not cause an immune response. Allograft as an alternative to autograft is the use of scaffold. The common bovine scaffolds that have been widely studied are DBBM (Deproteinized Bovine Bone Material), FDBB (Freeze-dried bovine bone xenograft) and dc-FDBB (Decellularized freeze-dried bovine bone xenograft). Permeability is a parameter that quantitatively measures the ability of a porous medium to flow liquids and depends on a combination of porosity, pore size, pore distribution and tortuosity. High viscosity can result in higher permeability. The porosity of decellularized cartilage bovine scaffold was significantly increased compared to fresh bovine cartilage.

Objectives to find out whether the level of permeability of dc-FDBB qualifies as a bone tissue engineering scaffold.

Scaffold FDBB, dc-FDBB, DBBM were divided into 2 groups, namely the group with distilled water and the group with 30% glycerol media. The permeability test was carried out in each group by measuring the liquid discharge every minute for 10 minutes and then calculating the permeability value. Then the permeability comparison between scaffolds is carried out. The maximum volume of fluid flow per minute was found on the DBBM scaffold, both in distilled water and 30% glycerol media. In the group with distilled water and 30% glycerol group, the highest permeability values were obtained in the DBBM scaffold test group, and the lowest in the dc-FDBB test group.

The permeability of the dc-FDBB scaffold in this study shows a value that falls within the range of human cancellous bone permeability values so that it can be concluded that the dc-FDBB scaffold in this study meets the scaffold permeability standards for tissue engineering.

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Introduction

Defects in the mandible can cause deformity, impaired function and facial esthetics which causes a decrease in a person's quality of life. Until now, mandibular defects are still a challenge for maxillofacial surgeons. Due to the limitations of traditional bone grafting, the development of modern regenerative medicine builds on engineering tissue design to recreate cells, tissues and guide the healing process.[1] Currently, there are various biomaterials used as scaffolds, both synthetic, inorganic and organic.[2] Bovine Scaffold which is common and has been widely studied is DBBM (Deproteinized Bovine Bone Material),[3] which is known to be difficult to degrade. The bovine scaffold was then processed using the lyophilization method (Freeze-drying) with FDBB (freeze-dried bovine bone xenograft) scaffold products. FDBB scaffold has the characteristics of being able to retain both inorganic and organic components so that it has good osteoconductive properties with little osteoinduction properties. Excess FDBB can be perfectly absorbed by the body so that it can support bone regeneration.[4,5,6]

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The important role in bone tissue engineering of the scaffold is greatly influenced by the characteristics of the scaffold. not only affects the mechanical properties but also affects the ability of a cell to penetrate the scaffold, the ability to diffuse nutrients, oxygen and metabolic products which ultimately affects revascularization and bone regeneration.[7] Permeability is a parameter that quantitatively measures the ability of a porous medium to flow liquids and depends on a combination of porosity, pore size, pore distribution and tortuosity.[8] In the previous permeability test study, namely the permeability test to human bone, several kinds of liquid media were used, namely aquades [9,10] and oil[11] where the viscosities were different. In the permeability research of the scaffold conducted by Lipowiecki, 30% water-glycerol was used as a simulation of the viscosity of the blood fluid.[10]

Objectives to examine the permeability of the freeze-dried bovine bone (FDBB) scaffold from decellularized cancellous bone (dc-FDBB) whether it is in accordance with the range of permeability values of cancellous bone in humans, as tissue engineering for bone regeneration in humans. mandibular defect.

Materials and methods

Research Samples

This research is an analytical observational study about the characteristics of biomaterials by comparing the permeability between scaffold groups. Production of samples of dc-FDBB, FDBB and DBBM at the Center for Biomaterials and the Network Bank of RSUD Dr. Soetomo, Surabaya. Experiments, data collection, data processing and compilation of results were carried out at the Research Center of the Faculty of Dentistry, Airlangga University, Surabaya.

Research Methods

1. Scaffold Preparation

The making of FDBB scaffold begins with taking pieces of cancellous bone in the bovine (bovine) region in the form of blocks with the maximum size that can be obtained. The bones are then soaked in a 3% hydrogen peroxide solution to remove any residual blood, fat, and bone marrow. Then rinsed with sterile distilled water to clean the remaining peroxide solution. After washing, the beef bones were then dried by freeze-drying at -80°C and dried with a lyophilizer until the moisture content was below 10%. The next process is packing in 2 layers or packing scaffold, tightly sealed and sent for sterilization using gamma rays. The stage of making the FDBB scaffold was produced by the network bank, RSUD Dr. Soetomo, Surabaya, Indonesia.

The decellularization method FDBB process must pass the decellularization stage using SDS and Triton X-100. The sample was allowed to stand at 4°C and then rinsed with phosphate saline (PBS) solution before being given SDS with a concentration of 0.5% for 1 hour. Samples were rinsed with sterile distilled water and then with PBS. The scaffold was then placed on a stirrer and rinsed with a solution containing chloroform and ethanol, with an initial ratio of 2:1 for 24 hours.

The DBBM manufacturing stage is produced by the network bank, RSUD Dr. Soetomo, Surabaya, Indonesia. The manufacture of this scaffold begins with taking pieces of cancellous bone in the bovine (bovine) region in the form of blocks with the maximum size that can be obtained. The bones were then washed with hydrogen peroxide, then washed with 0.9% NaCl. Then the defatting process to remove the remnants of fat. The deproteination process is carried out by burning at a temperature of 1000°C, then washing with sterile distilled water. Then dried in the oven at a temperature of 100°C. The next step is packing or wrapping the scaffold in two layers, tightly sealed and sent for sterilization using gamma ray radiation.

2. Permeability Test

Two kinds of fluids with different viscosities were used to test the permeability of the scaffold. The first liquid uses distilled water, the second liquid with 30% glycerol which has a higher viscosity as a simulation of blood viscosity. Preparation of the liquid for testing is carried out by heating the liquid to a temperature of 37^{O}C using a water bath, followed by measuring viscosity with an Ostwall viscometer and calculating the density of the liquid with a measuring cup and scales. The permeameter is designed with the constant head method consisting of a reservoir with flow into and out of the reservoir using a pump to maintain a constant water level (Figure 1). The reservoir is placed on top of the scaffold. The reservoir outlet is connected to a chamber made of acrylic with a 0.6 mm diameter pipe hose. The acrylic chamber is specially made to follow the shape of the scaffold to be tested, which functions to hold the scaffold during the testing process. The outlet at

the bottom of the chamber opens with outside air so that it has a pressure according to atmospheric pressure. At the bottom of the outlet of the chamber is placed a measuring cup to accommodate and measure the volume of liquid that passes through the scaffold during the test time.

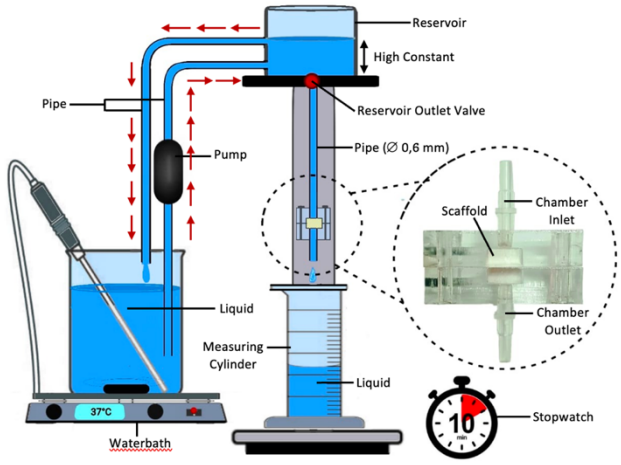

Figure 1. Permeater with constant head method.

The test was carried out for 10 minutes, starting with flowing liquid for 1 minute to remove air on the scaffold. The volume of water passing through the scaffold was measured every minute during the test time. From the measurements and tests, data were obtained for the value of the volume of water that is accommodated every minute, the cross-sectional area of the scaffold, the thickness of the sample, the height of the reservoir from the surface of the scaffold, the viscosity of the liquid, and the density of the liquid. From the data on the volume of water that is accommodated and the time of the test, the value of the water discharge is obtained. From the density data, reservoir height, and fluid density data, data on pressure changes can be obtained, which can then be calculated using the Darcy equation for the permeability value of the scaffold.

Statistical Methods

Statistical analysis used is Shapiro-wilk for normality test and Levene's Test for homogeneity test. Then the One-Way Anova test was carried out to see the difference in permeability between groups.

Results

The results of the average fluid volume per minute on the permeability test of the dc-FDBB, FDBB and DBBM scaffolds with distilled water and 30% glycerol can be seen in Figure 2. The volume of fluid flow per minute is mostly found on the DBBM scaffold both in distilled water and 30% glycerol media. The results of the comparison of the permeability test of the FDBB scaffold, dc-FDBB and DBBM with 30% glycerol can be seen in Figure 3. The permeability values for both distilled water and 30% glycerol media were found in the highest DBBM scaffold test group, and the lowest in the dc-test group. FDBB. In the test media using 30% glycerol, it was found that the permeability value of the scaffold was higher than the group with distilled water as the test medium.

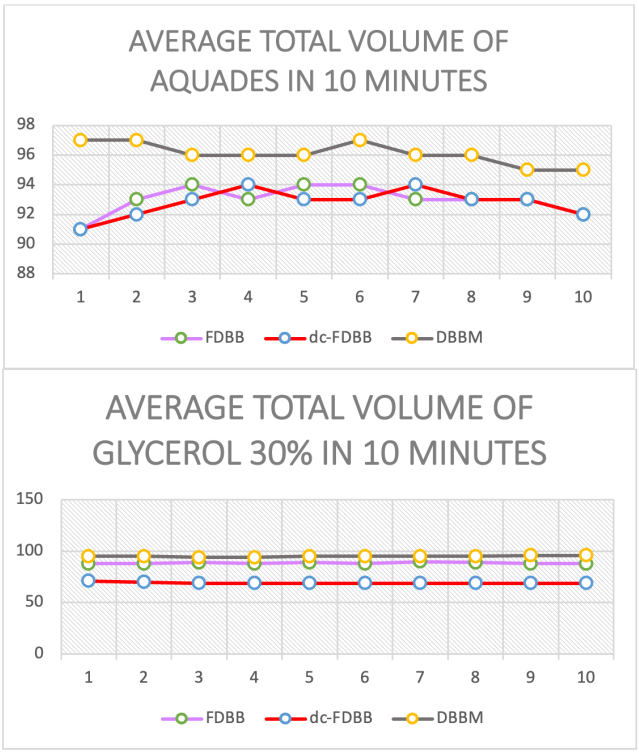

Figure 2. Comparison graph of the average total volume of liquid in 10 minutes.

The data distribution test was carried out using the Shapiro-Wilk test to show the significance value of p for the group. The p value > 0.05 indicates that the data from the permeability test results for each group is normally distributed. The homogeneity test carried out was Levene's test which showed a significance value of p = 0.007 where in this study the data were not homogeneous or heterogeneous. The results of the ANOVA test showed a significance value of p = 0.000 (p <

0.05), which means that there is a significant difference between groups. Post Hoc test using the Games-Howell test to see differences in the treatment groups. In the scaffold test group with distilled water and the scaffold test group with 30% glycerol media, there were no significant differences between the scaffold groups. When compared based on the group of media used, namely between the aquadest media group and the 30% glycerol media group, it was found that several scaffold test groups were significantly different, namely between the aquadest FDBB scaffold group compared to the 30% glycerol FDBB group and between aquadest DBBM group compared to the glycerol DBBM group 30%.

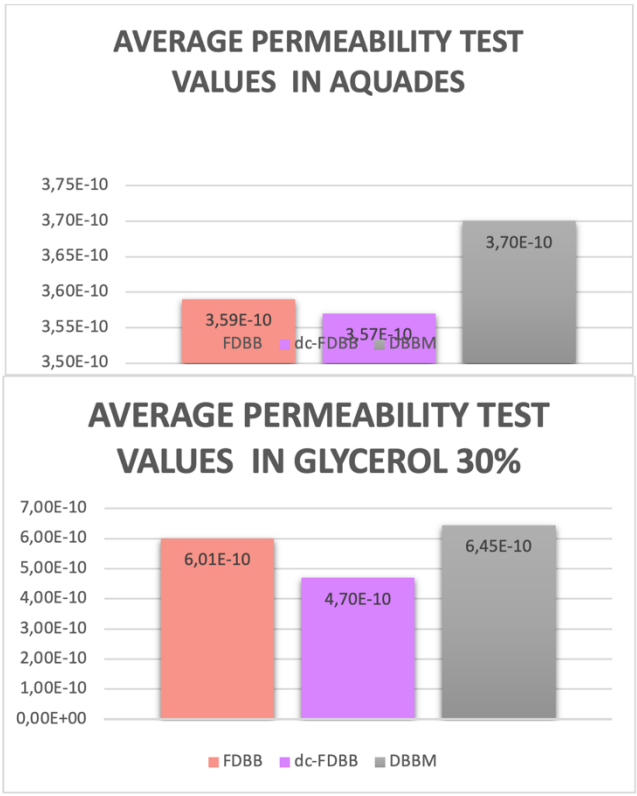

Figure 3. Graph of the comparison of the average permeability test values on FDBB, dc-FDBB and DBBM scaffolds with different viscosities.

Discussion

In this study, the permeability level test was carried out using the constant head method for a test time span of 10 minutes using liquid media with different viscosities, namely distilled water and 30% glycerol. The first liquid uses distilled water with a density (ρ) of 993.3 kg/m3 and a viscosity (μ) of 0.69 mpas at 37oC. The second liquid uses 30% glycerol, where at 37oC 30% glycerol has a density (ρ) of 881 kg/m3 and a viscosity (μ) of 1.08 mpas. Based on previous research, 30% glycerol was used as a liquid medium because it has a higher viscosity than distilled water and its viscosity is close to that of blood. Blood plasma has a viscosity ranging from 1 mPas to 1.3 mPas. The volume of fluid passing through the scaffold increases with the increase in the porosity and interconnectivity of the scaffold, this is due to the higher porosity and interconnectivity, providing space for higher fluid flows.[10]

The study begins with measuring the average value of the volume of fluid that passes through the scaffold within 10 minutes which is observed per minute. This observation resulted in the highest total fluid volume on the DBBM scaffold, and the lowest on the dc-FDBB scaffold. This is probably due to the different characteristics of each type of scaffold. At the time of making DBBM, the deproteination stage was processed by heating at a temperature of 1000°C. This can lead to the formation of a high level of porosity so that the DBBM obtains the highest total volume of liquid compared to dc-FDBB and FDBB. The level of porosity of a material can be affected by the temperature during the manufacturing process. In the manufacturing process with high temperatures above 400°C, the carbonate content of bone can be eliminated, thus affecting the density and porosity of a material. The carbonate content in bone cannot be eliminated properly if it is only processed below 400°C.[12] In a previous study conducted a study related to the relationship between temperature and porosity in bovine bone, in the temperature range of 600°C the porosity structure still did not show an increase, then at a temperature of 750°C - 900°C began to be seen an increase in the level of porosity, interconnectivity and Good porosity was obtained (±200 – 400 m) when processed at a temperature range of 900°C - 1200°C.[13,14] Therefore, in this observation of the total volume of liquid, the total volume of liquid in both aquadest and 30% glycerol media was the largest in the DBBM scaffold due to the high level of porosity.

Based on the results of the study, it was obtained that the permeability value was highest in the DBBM *scaffold* test group and the lowest in

the dc-FDBB test group. In data analysis with SPSS, there was no significant difference between the three materials. This correspondingly shows that the three materials basically have almost the same degree of permeability to each other. DBBM is made through a deproteination process where the process uses a temperature of 1000 °C which results in a high degree of porosity formed as explained earlier, thus causing the permeability value to also be higher than that of other scaffolds.[13,14]

In FDBB, *the scaffold* is processed through a *freeze dry* process. The *freezing* process with temperatures up to -80 °C results in the nucleation being at a low temperature, thus causing a homogeneous nucleation. This leads to the formation of more evenly distributed crystalline formations on the surface of the sample. On the other hand, when the *freezing* process is carried out at temperatures below -80 ° C, the nucleation is still not at a low temperature, causing heterogeneous or uneven nucleation on the surface of the sample.[15,16] This causes porosity and interconnectivity to form at -80°C, thus affecting the permeability of the *scaffold*.

In the dc-FDBB *scaffold*, the *scaffold* manufacturing process is the same as the FDBB manufacturing process as described above, what distinguishes this *scaffold* is that an additional decellularization process is carried out. This decellularization process aims to eliminate cellular components that may cause immune and inflammatory responses when *scaffolds* are applied.[17,18] Previous research stated that this decellularization process has a negative impact, namely causing some damage to the structure and composition of the *scaffold* material, in addition to the usefulness of this process can reduce the risk of immune and inflammatory responses of a *scaffold*. The SDS used in this process can cause some microstructure damage, namely in the lamellar structure and collagen. In the study, it was also reported that there was a slight decrease in the mechanical properties of *the scaffolds* tested after being decellularized using SDS materials. This can lead to a decrease in the elasticity of the *scaffold.*[19] Previous studies of well-decularized bone scaffolds used medium density (0.434 ± 0.015 mg/mm3) thereby obtaining balance in nutrient transport, cell attachment, cell infiltration and proliferation, matrix production, and *scaffold* mechanical strength.[20,21]

In this study, the dc-FDBB permeability value was obtained in accordance with the standard range of bone permeability values of human chancellors, although among other *scaffolds*, the lowest permeability value was obtained, but statistically there was no significant difference in the permeability value between the three *scaffolds*. In the previous study, it was stated that the standard permeability value of the bone *scaffold* is said to be ideal if it is in accordance with the range of bone permeability values of human chancellors, namely in the range of 1.2 x 10^{-10} to 539 x 10^{-10} where in the study used liquid media in the form of aquades.[9,22]

Conclusions

The permeability of the dc-FDBB scaffold (3.57 x 10^{-10} in aquadest media and 4.7 x 10^{-10} in glycerol media 30%) in this study shows values that fall into the range of values permeability of human chancellor bones (1.2x10^{-10}-539x10^{-10}) so it can be concluded that *scaffold* dc-FDBB in this study met the scaffold permeability standards for tissue engineering.

Ethical policy and institutional review board statement

Ethical clearance had been obtained from the Ethics Commission of the Faculty of Dental Medicine, Universitas Airlangga, Surabaya (No.624/HRECC.FODM/XII/2021), 2021.

Declaration of Interest

The authors declare that there are no conflicts of interest.

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