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Compressive Strength and Porosity Size of Bovine-Gelatin-Chitosan Hydroxyapatite Scaffold

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Abstract

Hydroxyapatite can also be obtained from synthetic and natural materials, such as bovine tooth hydroxyapatite for bone graft. Gelatin is a type-I collagen which is considered to be the main organic component in bone. Chitosan has the same structure as glycosaminoglycans (GAGs), non-collagenous organic components of bone.

This study aims to investigate the compressive strength and porosity of the HAGS-Gel-Kt scaffold with a ratio of 70:15:15 (w/w/w), Sigma-Aldrich Germany synthetic HA was used as comparison.

HAGS:Gel:Kt scaffold was made with a ratio of 70:15:15, made with a weight of 1.75 gr HAGS powder, 0.375 gr gelatin and 0.375 gr chitosan. The HAGS-Gel-Kt scaffold used for pore size testing is a scaffold with a diameter of 5 mm and a height of 5 mm. The procedure for measuring the size of the porosity is carried out using scanning electron microsope (SEM). The compressive strength test was carried out using diameter of 8 mm and a height of 17 mm scaffold examined with universal testing instrument.

HAGS-Gel-Kt cross-scaffold has higher size pore average than HAGS-Gel-Kt surface scaffold, HASyn-Gel-Kt surface scaffold, HASyn-Gel-Kt cross scaffold with significant different. HASyn-Gel-Kt has higher compressive strength than HAGS:Gel:Kt with significantly different ($p<0.05$). The compressive strength of the HAGS:Gel:Kt scaffold is equal to 0.221 MPa and the compressive strength of the HASyn:Gel:Kt scaffold is 0.370 MPa.

HAGS-Gel-Kt scaffold has good compressive strength and porosity size for bone tissue regeneration.

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Introduction

Autologous bone grafting is the gold standard procedure used to replace missing or defective bone. These grafts have disadvantages such as donor site morbidity, limited availability, and the need for secondary surgery.[1,2] The solution to overcome the bone tissue reconstruction using triad tissue engineering is tissue engineering which is carried out by combining three basic components: cells, scaffolds and regulatory signals.[3] The scaffold is a three-dimensional structure that is used as a temporary replacement for the damaged natural extracellular matrix (ECM), also providing a suitable microenvironment for the regeneration process of the damaged tissue.[4] The scaffold serves to support cell attachment and direct the growth of these cells into bone tissue, until the entire scaffold is replaced with new tissue.[1,5]

The mechanical properties of the scaffold are expected to be the same as the mechanical properties of normal bone. Mechanical properties can be measured by several parameters. The most widely used parameter is compressive strength. Compressive strength is the ability of a material to withstand pressure loads. Meanwhile, the porosity of the scaffolds is required for cell attachment. Porosity can also determine the mechanical properties of the scaffold. The size of the porosity of the scaffold can be observed

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using a scanning electron microscope (SEM). Biomimetic scaffolds made of hydroxyapatite, gelatin, and chitosan have been developed. Scaffold with hydroxyapatite, gelatin, and chitosan composition resembles ECM bone consisting of inorganic components (70%) and organic components (30%).[6] Hydroxyapatite is the largest inorganic component. Hydroxyapatite is osteoconductive and biocompatible, which is able to integrate well and firmly in bone.[7]

Hydroxyapatite can also be obtained from synthetic and natural materials, such as bovine tooth hydroxyapatite. Hydroxyapatite of bovine teeth has similarities with hydroxyapatite in humans.[8] Gelatin is a type-I collagen which is considered to be the main organic component in bone (90%). The main advantage of gelatin is that it increases cell attachment and growth because gelatin has many arginine-glycine-aspartic acid (RGD) protein chains and sequences. Gelatin is also biodegradable and biocompatible, but has poor biomechanical properties (1). Chitosan has the same structure as glycosaminoglycans (GAGs), non-collagenous organic components of bone. Chitosan is biocompatible, biodegradable, bioactive, and osteoconductive. Chitosan also has antimicrobial activity. Chitosan can help cell attachment, differentiation, and migration.[9] The integration of hydroxyapatite (HA), gelatin (GEL), and chitosan (K) on the scaffolding is expected to improve the mechanical and biological properties so that they can be considered as ideal scaffolds for bone regeneration. Scaffold hydroxyapatite, gelatin and chitosan with a ratio of 70:15:15 (w/w/w) are good biomaterial candidates for tissue engineering in bone.[10]

There has been limited recent study on bovine tooth hydroxyapatite scaffolds, gelatin and chitosan (HAGS-Gel-Kt). Therefore, this study focused on the utilization of hydroxyapatite derived from bovine teeth. This study aims to determine the compressive strength and porosity of the HAGS-Gel-Kt scaffold with a ratio of 70:15:15 (w/w/w), Sigma-Aldrich Germany synthetic HA was used as comparison. The HAGS-Gel-Kt scaffold is expected to be a biomaterial for the development of regenerative therapy for bone defects in dentistry.

Materials and methods

The materials used to make the scaffold are oxalic hydroxyapatite, chitosan powder with medium molecular weight (190,000-310,000 Da, 75-85% diastelation degree) (Sigma Aldrich 448877, USA), gelatin powder from cowhide (Sigma Aldrich G9391, USA), 0.1M NaOH (Biomedika), 2% acetic acid (Merck), and aqua distillate (Duta Farma).

HAGS:Gel:Kt scaffold was made with a ratio of 70:15:15, made with a weight of 1.75 gr HAGS powder, 0.375 gr gelatin and 0.375 gr chitosan. Chitosan that has been weighed is mixed with 4 ml of 2% acetic acid in a backer glass using a syringe, then stirred on a magnetic stirrer until the consistency thickens. The weighed gelatin was dissolved in aqua and then added to the chitosan solution which had thickened little by little until well mixed. The weighed HAGS powder was mixed with a mixture of gelatin and chitosan. The resulting mixture of HAGS, gelatin and chitosan that has been mixed, then added 0.1 M 1.9 ml of NaOH to neutralize the acid and if it is well mixed it is put into the mold and compacted so that there are no air voids that have not been filled. The mold is put into the freezer at 80°C for 2x24 hours and freeze-drying process is carried out for 2x24 hours. The method of making the scaffold in this study is a modification of the method of making the scaffold in the previous study.[11]

The HAGS-Gel-Kt scaffold used for pore size testing is a scaffold with a diameter of 5 mm and a height of 5 mm. The procedure for measuring the size of the porosity is carried out using SEM. The compressive strength test was carried out using a scaffold with a diameter of 8 mm and a height of 17 mm. The size of the scaffold used depends on the specifications of the tools used. The surface area of the scaffold to be used is measured first. The table on the autograph is covered with paper. The scaffold is placed in the center of the table with the vertical axis of the sample perpendicular to the plane. The autograft was turned on then the sample was pressed at a speed of 10 mm/min and the load cell was compressed by a 100 kN machine until the scaffold was distorted.

The instrument will then stop automatically and the numbers that come out are recorded.

The compressive strength value is then calculated using the following formula[12]:

Compressive strength = Force (Newton) / Surface area (mm²)

Results

The SEM examination was carried out on the HAGS-Gel-Kt scaffold and the HASyn-Gel-Kt scaffold on the surface and cross sections. In the SEM results, pore diameter measurements were carried out using the ImageJ program (Figure 1). The results of the diameter measurement in the ImageJ program were processed with the Excel program to obtain the average pore value (Table 1).

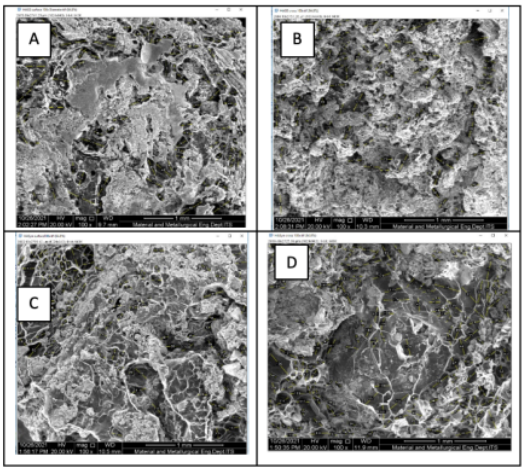

Figure 1. Size pore diameter measurement of SEM on HAGS-Gel-Kt surface scaffold (A), scaffold HAGS-Gel-Kt cross (B), HASyn-Gel-Kt surface scaffold (C), scaffold HASyn-Gel-Kt cross (D) at 100X magnification.

Measurement of pore diameter on SEM results used ImageJ program. ImageJ is a Java-based image processing program developed at the National Institutes of Health (NIH) and the Laboratory of Optical and Computational Instrumentation used to analyze microscopy images, area measurements, particle counting, segmentation and measurement of spatial or temporal features of biological elements. From the measurement results obtained the following results (Table 1).

	Surface		Cross	
	Mean	StDev	Mean	StDev
Scaffold HAGS-Gel-Kt	124.093	10.392	159.589	12.285
Scaffold HASyn-Gel-Kt	103.991	12.707	136.740	11.191

Table 1. Pore diameter (µm) scaffold HAGS-Gel-Kt surface, scaffold HAGS-Gel-Kt cross, scaffold HASyn-Gel-Kt surface, scaffold HASyn-Gel-Kt cross.

The difference in pores between scaffolds was carried out by normality test, it was obtained information that the significance of 0.00, ($p< 0.005$) indicated that the data distribution was not normal (Kolmogorov-Smirnov test) then the comparison test between variables was continued with the Kruskal Wallis H test which obtained a significant difference between the variables 0.000 $p< 0.05$, this indicates that there is a significant difference between the variables. Furthermore, to determine the difference between pairs of variables used the Mann Whitney test. The results of the Mann Whitney test obtained that the significance of all pairs > 0.05 indicated that all pairs of variables were significantly different (Table 2).

	Scaffold HAGS-Gel-Kt *Surface*	*Scaffold* HAGS-Gel-Kt *Cross*	*Scaffold* HASyn-Gel-Kt *Surface*	*Scaffold* HASyn-Gel-Kt *Cross*
Scaffold HAGS-Gel-Kt *Surface*		0.0001*	0.017*	0.049*
Scaffold HAGS-Gel-Kt *Cross*			0.0001*	0.008*
Scaffold HASyn-Gel-Kt *Surface*				0.0001*
Scaffold HASyn-Gel-Kt *Cross*				

Table 2. The results of the difference in pore diameters of the variable pairs scaffold of HAGS-Gel-Kt surface, HAGS-Gel-Kt cross, HASyn-Gel-Kt surface, HASyn-Gel-Kt cross.
*information: significant at $p<0.05$

The mean of the compressive strength of the HAGS-Gel-Kt scaffold and the HASyn:Gel:Kt scaffold shown in Figure 2. The results of the normal distribution of the One-Sample Kolmogorov-Smirnov Test obtained that the value of $p= 0.001$ ($p<0.05$) indicates that the data distribution is not normal. Furthermore, to determine the significance of the data differences, the Mann Whitney U test was used to obtain $p=0.00$ ($p<0.05$), which indicated that there was a significant difference between the average

compressive strength of the HAGS:Gel:Kt scaffold and the HASyn:Gel:Kt scaffold. The compressive value of 1 N/mm^2 is equal to 1MPa, meaning that the compressive strength of the HAGS:Gel:Kt scaffold is equal to 0.221 MPa and the compressive strength of the HASyn:Gel:Kt scaffold is 0.370 MPa, these values are still within the range that can be used on trabecular bone, namely 0.1- 16 MPa.[13]

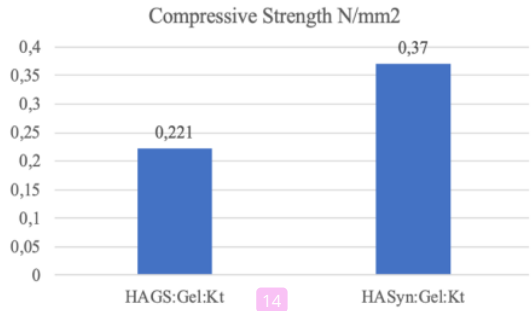

Figure 2. The mean of the compressive strength of the HAGS-Gel-Kt scaffold and the HASyn:Gel:Kt scaffold.

Discussion

The mechanical properties of the scaffold are a factor that must also be considered in the process of making scaffolds. The scaffold must be strong enough to withstand the mechanical stresses emanating from the surrounding tissue. Therefore, the low mechanical properties of the scaffolds can cause changes in the dimensions of the scaffolds. The compressive strength value in this study was 0.221 MPa, this value was still in the range that could be used on trabecular bone, namely 0.1-16 MPa.[13]

Another important property that must be possessed by a scaffold is the appropriate size of porosity. The size of the porosity is related to cell adhesion and migration as well as the diffusion of nutrients and the removal of metabolic wastes.[14] In this study, the average diameter of all samples had a pore size of more than 100 m, and the average porosity size of the HAGS-Gel-Kt scaffold was 124,093 m for the surface area and the median section was 159.589 m. This is in accordance with previous studies which stated that the minimum pore size of scaffolds was 100-150 m.[9] Suitable porosity sizes are used for mesenchymal stem cells (MSCs) attachments measuring 17.9-30.4 m.[15] The size of the porosity that is too small will cause limited cell migration and will interfere with the diffusion of nutrients and metabolic wastes. When this happens, it will cause scaffold necrosis. While the size of the porosity of the scaffold that is too large will cause the cells to be easily separated from the scaffold.[9,14]

Conclusions

It can be concluded that the HAGS-Gel-Kt scaffold has good compressive strength and porosity size values for bone tissue regeneration applications. However, further study is still needed to investigate the others mechanical, chemical and biological properties in vitro and in vivo study.

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Declaration of Interest

The authors report no conflict of interest.

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