Mechanical Evaluation of Anadara Granosa Scaffold with Various Gelatin Concentrations for Bone Regeneration

Meinar Nur Ashrin*[1], Widyasri Praningrum[2], Fitria Rahmitasari[2], Teofilus Timon Lirungan[1], Ryski Dea Citra Anindita[1], Rima Parwati Sari[3]

1. Prosthodontic Department, Faculty of Dentistry, Universitas Hang Tuah.
2. Dental Material Department, Faculty of Dentistry, Universitas Hang Tuah.
3. Oral Biology Department, Faculty of Dentistry, Universitas Hang Tuah.

Abstract

Various cases of bone defects needed bone graft as a substitute for damaged bone. Bone graft also acts as a scaffold or framework so that bone cell reparation occurs. The combination of Anadara granosa and gelatine can be used as an alternative of bone graft replacement for bone regeneration.

To evaluate the mechanical strength of Anadara granosa scaffold with variation of gelatine's concentrations.

The experiment was held completely randomized design divided into 3 groups. P1 group was scaffold Anadara granosa combination gelatine 5%, P2 group was 10%, and P3 group was 20%.

There was a significant difference (p=0,002) on diametral tensile strength between group. While the compressive strength of P3 group was significantly higher than other group (p=0,002).

There was a significant difference of mechanical strength of Anadara granosa scaffold with various gelatine concentrations.

Experimental article (J Int Dent Med Res 2021; 14(2): 514-518)

Keywords: Bone graft, Compressive strength, Diametral Tensile Strength, Anadara granosa.

Received date: 11 August 2020 **Accept date:** 10 January 2021

Introduction

Most bone defect (90%) is caused by tooth extraction, trauma and jaw diseases such as cysts or jaw tumors. Various cases of bone damage (bone defect) are often required bone graft in lieu of bone damaged [1]. Bone defect will have a bone healing process called bone healing. Bone healing consists of 3 phases, which are reparative, inflammation dan remodelling phase. Remodelling phase is the final process of bone healing [2]. Treatment using bone graft is used to stimulate bone formation and bone regeneration. Bone graft also acts as a scaffold or skeleton resulting in bone cell repair [3,4]. One of the therapies used to assist the process of bone formation and addition of bone height is bone graft. The bone graft material can be grouped into four types: *autograft*, *allograft*, *xenograft* and *alloplast* [5].

Requirements to be met by bone graft are acceptable to the body or biocompatible and favorable to the osteoconduction process (guiding the reparative growth of the natural bone), osteoinduction (inducing cells to differentiated into active osteoblasts), and osteogenesis (living bone cells in bone material graft that contributes to bone remodeling). The ideal bone graft is being available in large quantities, low prices, low toxicity and low morbidity, bioresorbable or degradable over an extended period of time, osteoconductively, osteoinductively, structurally similar to human bone, providing a good environment for passage through blood vessels and osteoblasts, have good mechanical properties, strong resistance, and adjustable to expected application. The conductivity of materials can be seen from the composition, the surface characteristics and the material's internal structure. Osteoconductive and osteointegration of bone graft related to porosity and pore size [6].

Scaffold is bone graft for bone regeneration. The scaffold can support the

Corresponding author:
Meinar Nur Ashrin,
Prosthodontic Department, Faculty of Dentistry, Universitas Hang Tuah, Jl Arif Rahman Hakim 150, Surabaya, Indonesia.
E-mail:meinar.ashrin@hangtuah.ac.id

process of growth and development of new tissue[7]. The main compound which present in the blood cockle shell is calcium carbonate ($CaCO_3$). Calcium carbonate is one of the biomaterials that has benefits for bone tissue regeneration applications as bone graft and Drug Delivery System (DDS) [8].

In addition, another material that can be used for bone graft is gelatine. Gelatine is a derived compound produced from collagen fibers of connective tissue hydrolyzed with acids or bases.[9] Gelatine is obtained from the hydrolysis of animal collagen contained in bone, and skin. Collagen as a natural biopolymer is able to provide specific signals for molecular interactions with cells that are sent specifically to specific tissue targets. Chicken shank collagen scaffold can increase VEGF expression at 2 weeks during bone regeneration.[10] Gelatine function is used for gel formation, stabilizers, thickeners, emulsifiers, crystallizers, foam-formers, coatings, adhesives, water binders, and purifiers . Other benefit from using gelatine is that it's easy manipulation in the mixing process of $CaCO_3$. Gelatine can also used in the making of scaffold preparation due to contribution to increase mechanical strength [11].

Scaffold must have solid mechanical strength in order to support the remodelling process of the bone. Mechanical strength can be tested by the Diametral Tensile Strength and compressive strength.

Gelatin is a natural product derived from partial collagen hydrolysis that proves to be biocompatible and stimulates cell proliferation and differentiation. In addition, the use of gelatin has been added in the form of a scaffold to mimic the composition of the bone.[11] In addition, the function of gelatin as a membrane barrier for bioactive molecules has a 3-dimensional and porous structure that can be attached to cells for tissue growth. Based on study of Sari et al (2020) , the addition of 10% gelatin in blood coockle shells containing CaCO3 significantly increased the number of osteoblasts and blood vessels compared to controls group.[12] Scaffolds with less gelatin content will result in less crosslink bonds between gelatin molecules making them weaker compared to scaffolds with more gelatin.

One of the mechanical characteristics of the scaffold needs to be don eto determine the ability to withstand the hydrostatic pressure of the tissue fluid and to maintain the space needed for cell growth and matrix formation, including through compressive and tensile stretngh tests.[13,14,15] Based on the above explanation, in this study the researchers wanted to know diametral tensile strength and compressive strength of scaffold from the shell of blood shells (*Anadara granosa*) with various gelatine concentrations.

Materials and methods

This research was a true experimental laboratory research. The design of this research was completely randomized design. Number of samples used were 24 samples divided into 3 groups. P1 was a scaffold group of *Anadara granosa* with 5% gelatine concentration. P2 was a scaffold group of *Anadara granosa* with 10% gelatine concentration. P3 was a scaffold group of *Anadara granosa* with 20% gelatine concentration.

Blood cockle shell sample preparation

The blood cockle shell was boiled for 30 minutes, then washed, brushed, and also cleaned on the outside and inside using water without bleach and then dried at room temperature. After that it was crushed using mortar and pestle to powdery form to make it easier to put in the cruissible for calcining process[12,16].

Blood Cockle shell calcination process

Firstly crussible and the lid was prepared and washed until it's clean. Weighed each empty crussible without lid marked. Then the cockle shell that has been prepared in the previous process was put into the crussible. Then all crussible were put into the furnace and set the temperature to 350^0C and time for 3 hours.[12]

Production Scaffold of Blood Cockle Shell with Variation Concentration of Gelatine using Freeze Dying Method

CaCO3 that was obtained from the blood cockle shell in powdery form was added to the gelation concentration and dissolved in a solvent (acetic acid or hydrochloric acid). Then CaCO3 and gelatine were molded into square-shaped. Next, CaCO3 and gelatine were put in the freezer with -50 degrees Celsius until it freezes. The last phase of freeze dry was the vacuum graft. It was inserted into the condenser. [12]

Diametral Tensile Strength Test

Diametral tensile strength or DTS test was measured by using *Universal Testing*

Machine (Shimadzu Autograph AG 5000 E) with *crosshead speed* = 5 mm/ menit and *Load* = 250 KgF. The specimen was put in the center diameter just below the load-feeding needle, then given the load and speed according to the instructions above until the specimen did not change shape. The number appearing on the monitor was then incorporated into the calculation formula with MPa unit. The DTS value (KgF/mm2) was converted into MPa. DTS (MPa) = DTS (KgF / mm2) x 9.80.[17]

Compressive strength test

In this study, compressive strength test used Universal Mechanical Testing Machine (Shimadzu Autograph AG 5000 E) with crosshead speed = 5 mm/min and Load = 250 KgF. The test sample form is usually cylindrical (6mm x 3mm) with the ratio of length and diameter (L/d) is 1 to 3.[17]

Formula for *compressive strength*:

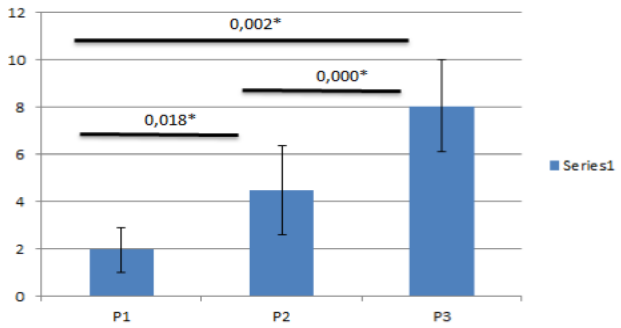
$$\sigma = \frac{F}{\pi r^2}$$

Where:
F= maximum load given on sample
Πr^2= sample cross-sectional area
Ϭ= pressure strength (Lb/mm^2).

Results

The data of the study was analyzed statistically.

Result of Diametral tensile strength test:

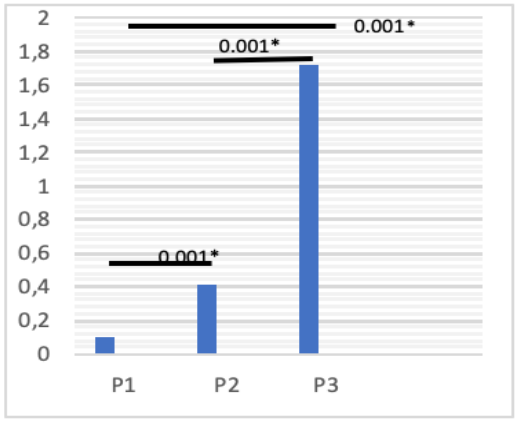


Graphic 1. Mean value and standard deviation of diametral tensile strength with LSD test.

Based on the data, the group with the highest mean was group 3 of 8,0583 Mpa, the group 2 4,4767 Mpa, and group 1 1,9600 Mpa.

The LSD Test results can be seen that the scaffold from the blood cockle shell (Anadara granosa) with gelatine showed that each group showed a significant difference between P1 and P3 i.e. 0,000 (p <0.05),
P1 and P2 groups were 0.018 (p <0,05), group P2 and P3 is 0,002 (p, 0 <05).

Result of compressive strength test*:*



Graphic 2. Descriptive Statistical Test Result of compressive strength with Mann-Whitney Test Result.

Based on the data, the group with the highest mean was group 3 of 1.722
Mpa, then the group 2 was 0.403 Mpa, and group 1 was 0.092 Mpa. Mann-Whitney test also shows that there were significant differences among all three treatment groups P1, P2, and P3.

Discussion

The aim of this research was to determine compressive strength of scaffold from Anadara shells (*Anadara Granosa*) and gelatine with variation of concentrations of 5%, 10%, and 20%.

From graphic 1, obtained an increase in the strength value of Diametral Tensile Strength scaffold material blood cockle shell (*Anadara granosa*). Diametral Tensile Strength in group P3, that was 8,0583 MPa with 20% gelatine concentration was the highest from the other. While the second sequence seen in group P2, that was 4.4767 MPa with 10% gelatine concentration. The lowest mean was obtained by P1 group, which was 1,9600 MPa with 5% gelatine concentration.

This results were possible because the mixing of $CaCO_3$ and gelatine caused the bond between R-COO-gelatine ions and Ca^{2+} ions

from $CaCO_3$. The crosslinking process caused the bonds between the gelatine molecules to become tight and frim with the $CaCO_3$ molecule. $CaCO_3$ is an inorganic bone-forming material, making it suitable to be used as a scaffold. The CO_3^{2-} ions of the $CaCO_3$ material is expected to substitutable with the phosphate groups on calcium phosphate ($CaPO_4$) in bone. The substitution of CO_3^{2-} ions in the phosphate group forms a type A apatite carbonate with the chemical formula $Ca_{10}(PO_4)_6CO_3$.

Ari (2018) said that in the concept of tissue engineering, biomaterial hydrophobicity properties is fundamental.[18] It has an important role in assisting cellular response. Gelatine is a derivative of collagen which is an essential part of bone, skin and connective tissue. Gelatine will be rapidly degraded by the enzymes so that the gelatine-based scaffold should be modified by the addition of other ingredients or increase crosslinking to slow its degradation. Mixing of $CaCO_3$ and gelatine can slow the degradation of scaffold $CaCO_3$. The tight bonds of gelatine molecules will hold $CaCO_3$ in the gelatine hydrogel. The content of gelatine will affect the crosslink bond between the gelatine molecules in a scaffold. Scaffolds with less gelatine content will affect the crosslink bonds between the fewer gelatine molecules making them weaker than those with higher gelatine concentrations. [9]

After LSD test results obtained $p < 0.05$ which shows differences in each group, then continued LSD test. This is to determine DTS in each group. Due to the fact that the higher the concentration of gelatine will produce stronger mechanical strength.

The synthetic bone graft should have an equivalent value of bone-like mechanical strength in order to withstand the burden of the body weight. One of the mechanical properties of the required bone replacement material is compressive strength and tensile strength.

Then, from graphic 2 obtained an increase in the strength value of DTS scaffold material blood cockle shell (*Anadara granosa*). DTS in group P3, that was 1.722 MPa with 20% gelatine concentration, is the highest from the other. While the second sequence seen in group P2, that is 0.403 MPa with 10% gelatine concentration. The lowest mean was obtained by P1 group, which was 0.092 MPa with 5% concentration. This is possible because the mixing of CaCO3 and gelatine causes the bonding between R-COO-gelatine ions and Ca2 + in CaCO3. So with more gelatine concentration bigger the increase of DTS will be.

Conclusions

Based on the result of this research, it can be concluded that there were significant differences of compressive strength and tensile strength values of scaffolds from *Anadara granosa* with different gelatine concentrations of 5%, 10%, and 20%. The highest scaffold compressive strength value is found on the scaffold with 20% gelatine concentration is 1,72 MPa, then the highest scaffold tensile strength value is found on the scaffold with 20% gelatine concentration is 8,0583MPa.

Acknowledgements

This research was supported by the Scientific Project Fund from Universitas Hang Tuah.

Declaration of Interest

The authors report no conflict of interest.

References

1. Darwis D, Warastuti Y. Sintesis dan karakterisasi Komposit Hidroksiapatit (HA) sebagai Graft Tulang Sintetik. J Ilm Apl Isot dan Radiasi. 2008;4(2):143-153.
2. Rodgers D. lecture 2: Fracture Repair. 2010;(September 2016):1-24. doi:10.1385/1-59259-863-3
3. Hardhani PR, Lastianny SP, Herawati D. Cangkok Tulang Terhadap Kadar Osteocalcin Cairan Sulkus Gingiva Pada Terapi Poket Infraboni. J PDGI. 2013;62(3):75-82.
4. Dumitrescu AL. Chemicals in Surgical Periodontal Therapy.; 2011. doi:10.1007/978-3-642-18225-9
5. Andrena A, Soeroso Y, Bachtiar EW. Evaluasi Pemberian Bahan Allograft dan Alloplast pada Penderita Periodontitis Agresif Menyeluruh dengan Genotipe Positif Alel 2 (+3954) Interleukin-1 Beta. J Dent Indones. 2013;15(2):135-140. doi:10.14693/jdi.v15i2.70
6. Develioglu H, Koptagel E, Gedik R, Dupoirieux L. The effect of a biphasic ceramic on calvarial bone regeneration in rats. J Oral Implantol. 2005;31(6):309-312. doi:DOI: 10.1563/1548-1336(2005)31[309:teoabc]2.0.co;2
7. Aufan MR, Daulay AH, Indriani D, Nuruddin A. Sintesis Scaffold Alginat-Kitosan-Karbonat Apatit Sebagai Bone Graft Menggunakan Metode. J Biofisika. 2012;8(1):16-24.
8. Hoque ME. Processing and Characterization of Cockle Shell Calcium Carbonate (CaCO3) Bioceramic for Potential Application in Bone Tissue Engineering. J Mater Sci Eng. 2013;02(04):2-6. doi:10.4172/2169-0022.1000132.
9. Pal K, Banthia AK, Majumdar DK. Polyvinyl alcohol-gelatin patches of salicylic acid: Preparation, characterization and drug release studies. J Biomater Appl. Published online 2006. doi:10.1177/0885328206056312

10. Pratiwi AR, Yuliati A, Ariani MD. Vascular Endothelial Growth Factor Expression after Induced by Chicken Shank Collagen Scaffold in Bone Regeneration. J Int Dent Med Res. 2017;10(2):333-337.
11. Xiaohua Liu, Smith LA, Hu J, Ma PX. Biomimetic Nanofibrous Gelatin/Apatite Composite Scaffolds for Bone Tissue Engineering. Biomaterials. 2009;30(12):2252-2258. doi:10.1038/jid.2014.371
12. Sari RP, Nurlaily I, Heryana RP, Fatmawati W AM. Journal of International Dental and Medical Research ISSN 1309-100X http://www.jidmr.com Anadara Granosa Shell-Stichopus Hermanni Rima Parwati Sari and et al. J Int Dent Med Res. 2020;13(2):525-532.
13. Sularsih, Soetjipto, Rahayu RP. The fabrication and characterization of chitosan-ethanol extracted aloe vera scaffold for alveolar bone healing application. J Int Dent Med Res. 2019;(4):1376-1381.
14. Brahatheeswaran Dhandayuthapani, Yasuhiko Yoshida, Toru Maekawa DSK. Polymeric Scaffold in Tissue Enginering Aplication: A Review. Int J Polym Sci. 2011;1(1):1-16.
15. Silva SS, Popa EG, Gomes ME, et al. An investigation of the potential application of chitosan/aloe-based membranes for regenerative medicine. Acta Biomater. 2013;9(6):6790-6797. doi:10.1016/j.actbio.2013.02.027
16. Setiawatie EM, Widiyanti P, Ryan M RM. Carbonate Hydroxyapatite-Hyaluronic Acid as Bone Healing Accelerator: in-vitro and in-vivo Studies on the Alveolar Bone of Wistar Rats. J Int Dent Med Res. 2019;12(4):1280-1286.
17. Feng P, Wei P, Shuai C PS. Characterization of mechanical and biological properties of 3-D scaffolds reinforced with zinc oxide for bone tissue engineering. PLoS One. 2014;9(1):e87755.
18. Muhammad Dimas Aditya Ari, Anita Yuliati, Retno Pudji Rahayu DS. The Differences Scaffold Composition in Pore Size and Hydrophobicity Properties as Bone Regeneration Biomaterial. J Int Dent Med Res. 2018;11(1):318-322.