The Differences Scaffold Composition in Pore Size and Hydrophobicity Properties as Bone Regeneration Biomaterial

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Abstract

Surgical procedure using bone replacement materials are still needed to accelerate new bone formation. Tissue engineering concept develop the research in scaffold biomaterial. Chitosan, gelatin and bovine hydroxyapatite combination can be synthesized as an ideal scaffold biomaterial that has a biomimetic properties of bone tissue.

To determine pore size and hydrophobicity properties of chitosan-gelatin/bovine hydroxyapatite scaffold at various eligible ratios in bone tissue engineering

Scaffold chitosan-gelatin/bovine hydroxyapatite with a ratio of 20:80, 30:70 and 40:60 synthesized using freeze dry method. Scaffold on each ratio was tested by pore size examination using Scanning Electron Microscope. The ratio of swelling and water content percentage was done by measuring the initial weight and final weight after being soaked in distillate water for 1, 3 and 7 days.

The smallest pore size was obtained at a 20:80 ratio scaffold with a mean value of 254.44 ± 37.96 μm and the largest on a 40:60 ratio scaffold with a mean value of 423.04 ± 68.72 μm. Swelling ratios and water content percentage were highest on the chitosan-gelatin/bovine hydroxyapatite ratio of 40:60 at day 7 (2,904 ± 0.531 and 75.84 ± 2.6%).

The pore size and hydrophobicity properties corresponding to bone tissue regeneration biomaterials were obtained on the 20:80 and 30:70 ratios chitosan-gelatin/bovine hydroxyapatite scaffold.

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Introduction

The development of biomaterials and cell therapy has reached to the development of tissue engineering concepts. This concept further encourages the research and use of scaffold biomaterials that can provide an extracellular matrices-like structure that allows cells to attach and proliferate.[1,2] One of the requisite key to the tissue engineering is that the scaffold must have such a biomimetic structure in the micro environment to allow regeneration. The most common regenerated tissue using biomaterials is bone. Surgery intervention using bone replacement materials are still needed to accelerate new bone formation.[1,3]

Chitosan is a polysaccharide that structurally similar to glycosaminoglycans, which is a major component of the extracellular matrix of bone and cartilage.[4] The advantage of Chitosan are low immunogenic potential, bioactive properties and has a good interaction with the host tissues, abundant availability in nature and has a potential effect in osteogenesis.[2,5] Hydroxyapatite have been widely used as biomaterials to replace and repair of bone tissue as it is one of the largest inorganic bone-building materials and good biocompatibility.[6] Gelatin as a polymer derived from natural materials has biopolymer properties such as biodegradable, biocompatible, and nontoxic.[7] Scaffold with a combination of chemical properties of chitosan, gelatin, and

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hydroxyapatite has the potential form to be a scaffold with good physical properties.[8]

The scaffold porous size and hydrophobicity properties are important to support the biomimetic properties in the tissue engineering concept. The hydrophobicity properties can be tested using swelling ratio and water content percentage. The pore size and hydrophobicity properties are important to support stem cells attachment into the bone regeneration biomaterials. The purpose of this study was to determine the pore size and hydrophobicity properties of chitosan-gelatin-bovine hydroxyapatite (Ch-G/BHA) scaffolds at various eligible ratios in bone tissue engineering.

Materials and methods

This research used chitosan with deacetylation degree > 80% (Sigma 93646, USA), gelatin (Rousselot, Ghuangdong China) and bovine hydroxyapatite sized 150-355 μm, was produced by RSUD Dr. Soetomo, Surabaya.

Scaffold synthesis

The scaffold fabrication procedure was done according the previous study[9] and has been modified to get the different eligible ratios. Scaffold with 30:70 ratios was made by mixing 0.375 gr gelatin and 2 ml acetic acid 2% and stirred on the magnetic stirrer until become gel. 1.75 gr BHA was wetted with deionized distillated water and mixed into the gel. The solution then was stirred until homogen. 0.375 gr chitosan was added then 9 ml NaOH 0.1M. To make 20:80 ratios scaffold, 0.25 gr gelatin, 2 gr BHA and 0.25 gr chitosan and for 40:60 ratios scaffold 0.5 gr gelatin, 1.5 gr BHA and 0.5 gr chitosan were needed. All mixtures then were placed into the mold and frozen on the -40ºC for 2x24 hours and continued *freeze drying* for 2x24 hour.

Scaffold pore size examination

Scaffold Ch-G/BHA coated with sputter coater then vacuum for 30 minutes. After the vacuum was done with plasma coating for 3 seconds using Au and Pb, Scaffold then undergone Scanning Electron Microscope (SEM) machines (Zeiss, Germany) at 100x magnification. Then the data were collected.[10]

Swelling ratio and water content percentage examination

The scaffolds in each ratio were weighed as initial weight (Wi). Scaffold was immersed in distillated water and incubated at 37ºC. Final weighing (Wf) was performed on the 1st, 3rd, and 7th days to get swelling ratio (SR) and water content percentage (WCP)[11]. Data obtained through calculating Wi and Wf in the formula:

$$SR = \frac{Wf - Wi}{Wi}$$

$$WCP(\%) = \frac{Wf - Wi}{Wf} \times 100\%$$

Statistical analysis

The scaffold pore size, SR and WCP data are shown in the mean value and standard deviation. The data were analyzed using One Way ANOVA on SPSS software version 15.0 (SPSS, Inc., Chicago, IL, USA). The p value <0.05 was considered as statistically significant.

Results

Scaffold synthesis

Scaffold Ch-G/BHA can be seen in figure 1.

Figure 1. Scaffold Ch-G/BHA.

Scaffold Pore Size

The result of SEM analysis is shown in figure 2. The pore size is then calculated and the average pore size of the scaffold Ch-G/BHA is shown in Figure 3. The largest porous sizes were obtained in the group of scaffolds Ch-G/BHA 40:60 of 423.04 ± 68.72 μm followed scaffolds Ch-G/BHA 30:70 of 311.82±59.86 μm and the smallest in the group scaffold Ch-G/BHA 20:80 amounted to 254.44 ± 37.96 μm. There was a significant difference in scaffold pore size between ratio 20:80 with 40:60 and 30:70 with 40:60 ($p < 0.05$).

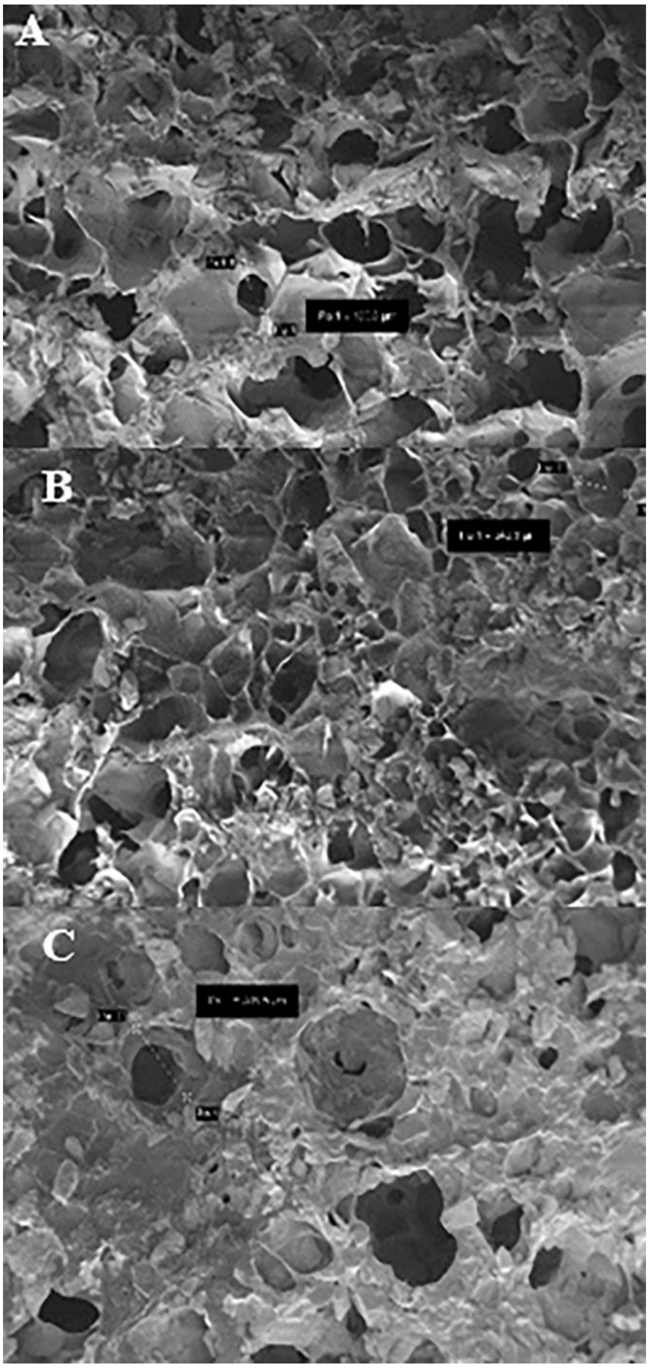

Figure 2. SEM result of scaffold Ch-G/BHA.
(A) Scaffold Ch-G/BHA 40:60. (B) Scaffold Ch-G/BHA 30:70.
(C) Scaffold Ch-G/BHA 20:80.

Swelling ratio and water content percentage

SR mean values (Figure 4) showed an increase in the duration of the study across all groups. Scaffolds Ch-G/BHA 40:60 showed the highest SR of 1.902±0.464; 2.193±0.533; 2.904±0.531 and scaffolds Ch-G/BHA 20:80 showed the lowest SR of 0.929±0.118; 1.177±0.154; 2.11±0.158 on the 1st, 2nd and 3rd day. There was a significant difference in swelling ratio between ratio 20:80 with 30:70 on 1st day and ratio 20:80 with 40:60 on 1st, 3rd and 7th day ($p < 0.05$).

The mean value of WCP also showed an increase by the duration of the study across all groups (Figure 5 On the three different time periods, the scaffold Ch-G/BHA 40:60 showed the highest WCP of 68.21±2.17%; 70.51±1.87%; 75.84±2.6% and the scaffold Ch-G/BHA 20:80 showed the lowest WCP of 48.03±312%; 53.88±3.12; 67.78±1.62 on the 1st, 2nd and 3rd day. There was a significant difference in water content percentage between ratio 20:80 with 30:70 on 1st and 3rd day, ratio 30:70 with 40:60 on 1st, 3rd and 7th day, and ratio 20:80 with 40:60 on 1st, 3rd and 7th day ($p < 0.05$).

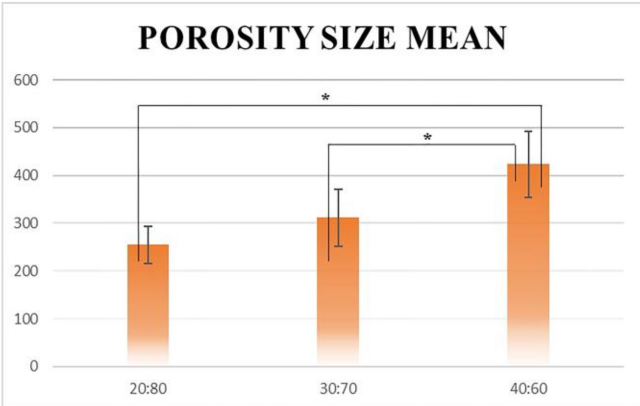

Figure 3. Mean value of *scaffold* Ch-G/BHA pore size in different ratio (μm).
*showed there was a significant statistical difference (p<0.05).

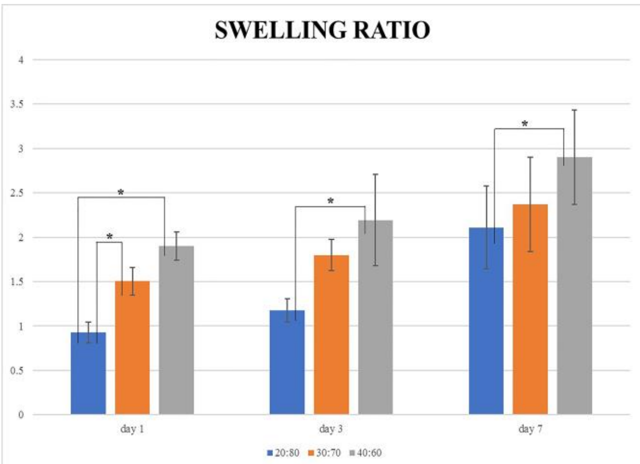

Figure 4. SR mean value of scaffold Ch-G/BHA in different ratio.
*showed there was a significant statistical difference (p<0.05).

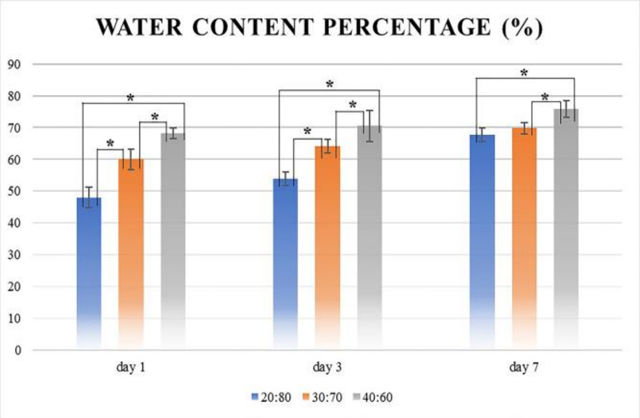

Figure 5. WCP mean value of scaffold Ch-G/BHA in different ratio.
*showed there was a significant statistical difference (*p*<0.05).

Discussion

In the last few decades, tissue engineering has developed rapidly and focuses on the reconstruction and regeneration of defect tissue and injury. In the concept of bone engineering, the most important thing is to induce stem cells to attach to biomaterials that have porosity to support the formation of new bone tissue.[12]

It has been widely studied that porous scaffolds provide a place for cells attachment, promote new tissue proliferation, vascularization and transport nutrients to attached cells and provide stable osteointegration and stable long-term fixation.[13-16]

Scaffold Ch-G/BHA is a good biomaterial candidate for bone tissue engineering. This is in accordance with the suitability of biomimetic properties of porous size and hydrophilic scaffold properties that have been done in this study. The scaffold pores in this study were obtained through the freeze-drying method by removing all solvents contained in the scaffold for 2x24 hours. The smaller porous signifies the presence of the dominant electrostatic forces among the polymer molecules, calcium ions and phosphate groups contained in hydroxyapatite and gelatin.[17]

In this study the addition of hydroxyapatite is expected to decrease the pore size of the scaffold. This result is supported by several studies which reveal that the addition of hydroxyapatite amounts in the scaffold composition causes the scaffold less porous more complex.[17,18] The study supports the result in this study that the largest pore size was found in the lowest hydroxyapatite ratio.

Studies have shown that in the concept of bone tissue engineering, the ideal pore size on scaffolds ranges from 200-350μm. This is an optimum pore size that allows osteoblast stem cells to form new bone tissue. Scaffold pore size also allows the induction of the formation of new blood vessels so that the nutritional needs of cells to form a new bone tissue to be optimal.[19-21] The pore sizes that are too small or too large cause the limitations of the distribution and migration of osteoblast stem cells in the scaffold.[1]

Biomaterial hydrophobicity can be measured using swelling ratio and water absorption through water content percentage. There is a synergic result of the research between swelling ratio with water content percentage. The highest score was obtained in the scaffold Ch-G/BHA 40:60 on the 7th day. SR and WCP were affected by microstructure and hydrophobicity of the scaffold biomaterial.[22] Gelatin is widely known as a biomaterial that has hydrophilic properties. Gelatin is able to absorb water up to 5 times compared to its dry weight.[23] This theory is in accordance with the results obtained in this study that the increase in SR and WCP is directly proportional to the increase in the amount of gelatin.

The higher hydroxyapatite ratio in the scaffold mixture leads to a decrease in SR and WCP. This is in line with previous study which revealed that the increase in the amount of hydroxyapatite is inversely proportional to the swelling ratio.[24] The hydrophobicity properties of biomaterials is very important in the concept of tissue engineering. The hydrophobicity of the biomaterial surface has an important role in regulating cellular attachment cell response. Biomaterials that have hydrophilic properties can improve the attachment of bone stem cells so as to accelerate the regeneration process of bone defects.[25,26]

Conclussions

From the results of this study, it can be concluded that the pore size and hydrophobicity properties corresponding to bone tissue regeneration biomaterials are obtained on the scaffold Ch-G/BHA ratios 20:80 and 30:70.

Declaration of Interest

The authors report no conflict of interest.

References

1. Murphy CM, O'Brien FJ, Little DG, Schindeler A. Cell-scaffold interactions in the bone tissue engineering triad. *Eur Cells Mater*. 2013; 26: 120-132.
2. Chen Y, Huang Z, Li X, Li S, Zhou Z, Zhang Y, Feng QL, Yu B. In vitro biocompatibility and osteoblast differentiation of an injectable chitosan/nano-hydroxyapatite/collagen scaffold. *J Nanomater*. 2012; 2012: 1-7.
3. Julia V, Maharani DA, Kartasasmita RE, Latief BS. The use of coral scaffold in oral and maxillofacial surgery: a review. *J Int Dent Medica Res*. 2016; 9: 427-36.
4. Thein-Han WW, Misra RDK. Biomimetic chitosan–nanohydroxyapatite composite scaffolds for bone tissue engineering. *Acta Biomater*. 2009; 5(4): 1182-1197.
5. Soeroso Y, Bachtiar EW, Boy BM, Sulijaya B, Prayitno SW. The prospect of chitosan on the osteogenesis of periodontal ligament stem cells. *J Int Dent Med Res*. 2016; 9(2): 93-7.
6. Bahrololoom ME, Javidi M, Javadpour S, Ma J. Characterisation of natural hydroxyapatite extracted from bovine cortical bone ash. *J Ceram Process Res*. 2009; 10(2): 129-138.
7. Link DP, van den Dolder J, van den Beucken JJ, Wolke JG, Mikos AG, Jansen JA. Bone response and mechanical strength of rabbit femoral defects filled with injectable CaP cements containing TGF-β1 loaded gelatin microparticles. *Biomaterials*. 2008; 29(6): 675-682.
8. Wattanutchariya W, Changkowchai W. Characterization of porous scaffold from chitosan - gelatin/hydroxyapatite for bone grafting. *Proc Int MultiConference Eng Comput Sci*. 2014; II: 1-5.
9. Kartikasari N, Yuliati A, Listiana I, Setijanto D, Suardita K, Ariani MD, Sosiawan A. Characteristic of bovine hydroxyapatite-gelatin-chitosan scaffolds as biomaterial candidate for bone tissue engineering. *IECBES 2016 - IEEE-EMBS Conf Biomed Eng Sci*. 2017: 623-626.
10. Yuliati A, Kartikasari N, Munadziroh E, Rianti D. The profile of crosslinked bovine hydroxyapatite gelatin chitosan scaffolds with 0.25% glutaraldehyde. *J Int Dent Med Res*. 2017; 10: 143-148.
11. Tangsadthakun C, Kanokpanont S, Sanchavanakit N, Banaprasert T, Damrongsakkul S. Properties of collagen chitosan scaffolds for skin tissue engineering fabrication of collagen/chitosan scaffolds. *J Met Mater Miner*. 2006; 16(1): 37-44.
12. Melek LN. Tissue engineering in oral and maxillofacial reconstruction. *Tanta Dent J*. 2015: 1-13.
13. Murphy CM, O'Brien FJ. Understanding the effect of mean pore size on cell activity in collagen-glycosaminoglycan scaffolds. *Cell Adhes Migr*. 2010; 4(3): 377-381.
14. Xiao X, Wang W, Liu D, Zhang H, Gao P, Geng L, Yuan Y, Lu J, Wang Z. The promotion of angiogenesis induced by three-dimensional porous beta-tricalcium phosphate scaffold with different interconnection sizes via activation of PI3K/Akt pathways. *Sci Rep*. 2015; 5(1): 1-11.
15. Janse van Rensburg A, Davies NH, Oosthuysen A, Chokoza C, Zilla P, Bezuidenhout D. Improved vascularization of porous scaffolds through growth factor delivery from heparinized polyethylene glycol hydrogels. *Acta Biomater*. 2017; 49: 89-100.
16. Wu S, Liu X, Yeung KWK, Liu C, Yang X. Biomimetic porous scaffolds for bone tissue engineering. *Mater Sci Eng R Reports*. 2014; 80(1): 1-36.
17. Kim H, Knowles JC, Kim H. Hydroxyapatite and gelatin composite foams processed via novel freeze-drying and crosslinking for use as temporary hard tissue scaffolds. *J Biomed Mater Res*. 2004; 72(2): 136-45.
18. Landi E, Valentini F, Tampieri A. Porous hydroxyapatite/gelatine scaffolds with ice-designed channel-like porosity for biomedical applications. *Acta Biomater*. 2008; 4(6): 1620-1626.
19. Rouwkema J, Rivron NC, Blitterswijk CA. Vascularization in tissue engineering. *Cell Press*. 2008; 6: 434-41.
20. Murphy CM, Haugh MG, O'Brien FJ. The effect of mean pore size on cell attachment, proliferation and migration in collagen-glycosaminoglycan scaffolds for bone tissue engineering. *Biomaterials*. 2010; 31(3): 461-466.
21. Rosdiani AF, Widiyani P, Rudyarjo DI. Synthesis and characterization biocomposite collagen-glycerol as a scaffold for gingival recession therapy. *J Int Dent Med Res.* 2017; 10(1): 118-22
22. Huang Y, Zhang X, Wu A, Xu H. Injectable nano-hydroxyapatite (n-HA)/glycol chitosan (G-CS)/hyaluronic acid (HyA) composite hydrogel for bone tissue engineering. *RSC Adv*. 2016 ;6(40): 33529-33536.
23. Yu TC, Yeh ML. Fabrication of cellulose-gelatin based endothelialed vascular graft with SMCs/ADSCs seeding in bioreactor. *IFMBE Proc*. 2015; 52: 35-38.
24. Dey S, Pal S. Evaluation of collagen-hydroxyapatite scaffold for bone tissue engineering. *13th Int Conf Biomed Eng*. 2009; 23(1-3): 1267-1270.
25. Goddard JM, Hotchkiss JH. Polymer surface modification for the attachment of bioactive compounds. *Prog Polym Sci*. 2007; 32(7): 698-725.
26. Xu LC, Siedlecki CA. Effects of surface wettability and contact time on protein adhesion to biomaterial surfaces. *Biomaterials*. 2007; 28(22): 3273-3283.