Characteristic of Bovine HydroxyapatiteGelatin-Chitosan Scaffolds as Biomaterial Candidate for Bone Tissue Engineering

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Characteristic of Bovine Hydroxyapatite-Gelatin-Chitosan Scaffolds as Biomaterial Candidate for Bone Tissue Engineering

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***Abstract* -- Bone defect is still a major problem in dentistry. Solving this problem, tissue engineering becomes one of the promising methods for bone regeneration. One of main component of tissue engineering is scaffolds. Hydroxyapatite (HA) and natural polymer are widely used for scaffolds in bone tissue engineering due to similarity to the natural bone. In this study, the author prepared the scaffolds from bovine hydroxyapatite (BHA), gelatin (GEL) and chitosan (K). The prepared scaffolds were analyzed by using FTIR and SEM-EDX. In addition, its swelling and degradation rate are also included in this analysis. The results are (1) FTIR reveals the compositions of the scaffolds, and it consists of BHA, GEL, and K; (2) SEM-EDX reveals the elements of the scaffolds, and it consists of carbon (C), oxygen (O), natrium (Na), magnesium (Mg), phosphor (P), and calcium (Ca); (3) the scaffolds' swelling rate is good due to its hydrophilic composition; (4) and the degradation rate is low within three days. The four results show that the BHA-GEL-K scaffolds could be one of biomaterial candidate for bone tissue engineering.**

Keyword: scaffolds, BHA-GEL-K, FTIR, SEM-EDX, swelling, degradation

I. INTRODUCTION

Bone defect is still a major problem in dentistry. Bone defect could be caused by degeneration disease, aggressive periodontitis, fracture, bone resection, and congenital defect [1]. In a small defect, bones are able to remodeling and healing a short time, whilst in a large defect (massive bone defect), it could cause a serious damage that it would be hard to recover [2].

In this case, surgical and bone reconstruction is needed. Bone Regeneration Graft (BGR) is one of the most well-known methods for bone regeneration but this method has several disadvantages. The approach of tissue engineering is considered as a promising method to regenerate bone defect. Tissue engineering has three basic components: cell, scaffolds, and regulator signal. These components are called triad tissue engineering [3]. Scaffolds are a three-dimensional structure used as a temporary substitution of Extra Cellular Matrix (ECM). Scaffolds might support attachment, proliferation, migration and differentiation of cells and regulator signal so bone regeneration might occur [4,5].

Recently, scientists began to develop biomimetic scaffolds made from hydroxyapatite (HA), gelatin (GEL) and chitosan (K) (HA-GEL-K). Scaffolds made of these materials are expected to resemble ECM in bone, which consists of 70% inorganic component (HA) and 30%organic components (Gel and K) [6]. HA is a major of inorganic component of bone. HA has been reported as a bioactive, biocompatible, osteoconductive, non-toxic and non-inflammatory biomaterial which induces cell proliferation and osteogenic differentiation. HA could be obtained from synthetic and natural materials. HA could be found from cow bone (bovine hydroxyapatite / BHA). BHA is a non-toxic material that has similarities with HA in humans [7].

Gelatin (GEL) is a biomaterial derived from collagen. Gelatins contain collagen type-I which is a major (90%) organic component of ECM [8]. This material has been reported as biodegradable and biocompatible material. Gelatin is composed from amino sequence such as arginine-glycine-aspartic acid (RGD) which promote cell adhesion, migration, and differentiation, and proliferation [9].

Chitosan (K) is a biopolymer product of chitin deacetylation. This material has the same structure as glycosaminoglycan (GAG) which is a non-collagen organic component of ECM. Chitosan is considered as an appropriate material for biomedical applications due to its biocompatibility, biodegradable, bioactivity, osteo-conductive, and anti-microbial activity. Chitosan might also support cell attachment, differentiation, and migration [10]. Combination of BHA, GEL, and K in form of scaffolds are expected to improve mechanical and biological properties.

In this study, the author prepared scaffolds from BHA, GEL, and K with compositions of 70:15:15 (w/w/w).The prepared scaffolds were analyzed by using FTIR and SEM-EDX. In addition, its swelling and degradation rate are also included. These scaffolds are expected to be a biomaterial candidate for bone tissue engineering.

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II. MATERIAL AND METHODS

A. Materials

BHA (particle size at<150 µm from cow bone) was purchased from Tissue Bank at RSUD Dr Soetomo, Surabaya. Gelatin was purchased from Rousselot, China. Chitosan (deacetylation degree >81%) was purchased from Sigma.

B. Preparation of scaffolds BHA-GEL-K

9 ml of 2% acetic acid and 0.375 gr gelatin were mixed using magnetic stirrer in 100ºC. BHA 1.75 gr was mixed with 5 ml distilled water. Then, the water was drained. The BHA mixture was mixed to gelatin and acetic acid mixture. Chitosan 0.375 gr and 2 ml of 10% NaOH were added. The mixture was put into a scaffolds's mold and frozen at -80°C for 24 hours and then it was freeze-dried for 2 x 24 hours.

C. Fourier Transform Infrared Spectroscopy (FTIR)

BHA, gelatin, chitosan, and scaffolds BHA-GEL-K were obtained by using FTIR Thermo Scientific, Nicolet, iS10. The analysis of sample used 400-4000 $cm^{-1}$of wavenumber.

D. SEM-EDX (Scouldning Electron Microscopy coupled with Energy Dispersive X-ray)

SEM-EDX was obtained by using FEI, Inspect-S50. The samples were coated with Au and Pb.

E. Swelling Studies

Swelling studies might determine its swelling ratio and water content percentage (WCP). BHA-GEL-K scaffolds were measured to determine initial weight (Wi). BHA-GEL-K scaffolds were soaked with distilled water for 24 hours at 37°C. After 24 hours, the sample was re-measured to determine its final weight (Wf). Its swelling ratio could be calculated using equation (1) and WCP could be calculated using equation (2) [11].

$$Swelling\ ratio = \frac{Wf - Wi}{Wi} \quad (1)$$

$$WCP\ (\%) = \frac{(Wf-Wi)\ x\ 100\%}{Wf} \quad (2)$$

F. Degradation studies

BHA-GEL-K scaffolds were measured to determine initial weight (Wi). BHA-GEL-K scaffolds were soaked with distilled water, which contains 1µg/ml (200 unit/ml) lysozyme for 3 days at 37°. The sample then were rinsed with distillated water and frozen at -80 °C for 24 hours. Freeze dry was conducted for 2 x 24 hours. After freeze-drying, the scaffolds were measured again to determine its final weight (Wf). Its degradation rate could be calculated by using equation (3) [12].

$$Degradation\ rate\ (\%) = \frac{(Wf-Wi)}{Wf} \times 100\% \quad (3)$$

III. RESULT AND DISCUSSION

BHA-GEL-K scaffolds, made of bovine hydroxyapatite, gelatin, chitosan, NaOH, and acetic acid,are shown in Fig. 1.

Fig. 1. Result of scaffolds making process using freeze dry method

A. FTIR

The spectrum of FTIR from BHA, gelatin, chitosan, and scaffolds BHA-GEL-K is shown in Figure 2. The result shows that there were some peaks of wavenumber at 407,68 cm^{-1}, 562,09 cm^{-1}, 598,37 cm^{-1}, 627,34 cm^{-1}, 961,11 cm^{-1}, 1024,97 cm^{-1}, 1086,65 cm^{-1}, 1406,07 cm^{-1}, 1545,78 cm^{-1},1636,98 cm^{-1}, and 3284,23 cm^{-1}.

In Fig 2., the red arrow indicates that there was phosphate (PO_4^{3-}) group from BHA in BHA-GEL-K scaffolds. PO_4^{3-} showed the wavenumber at 560-600 cm^{-1} and 1000 – 1100 cm^{-1} [13]. The result FTIR of BHA showed the peak of wavenumber at 564,67 cm^{-1}, while BHA-GEL-K scaffolds at 562,09 cm^{-1}.PO_4^{3-} group could be bond with COO^- and NH_3^+group from GEL and K [14].

Blue arrow indicates that there were amide A and amide II group from gelatin in BHA-GEL-K scaffolds. Amide A showed the wavenumber at 3600-2300 cm^{-1} while amide II at 1560-1335 cm^{-1}[15]. The result of FTIR of GEL indicates that amide A showed the peak of wavenumber at 3275,85 cm^{-1}, while BHA-GEL-K scaffolds at 3284,23cm^{-1}. The result of FTIR of GEL indicates that amide II showed the peak of wavenumber at 1541,15 cm^{-1}, while BHA-GEL-K scaffolds at1545,78 cm^{-1}.

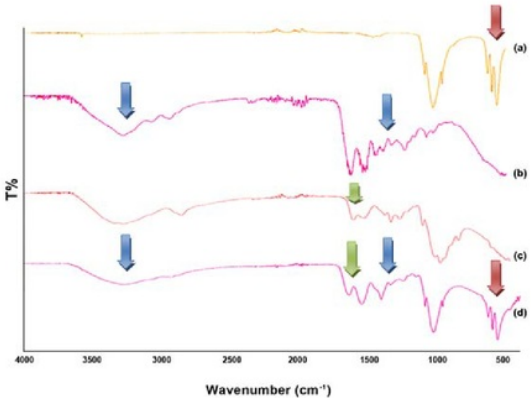

Fig. 2. FTIR spectrum of BHA (a), gelatin (b), chitosan (c), and scaffolds BHA-GEL-K (d)

Green arrow indicates that there was amino group of chitosan in BHA-GEL-K scaffolds. Amine showed the wavenumber at 1650-1580 cm^{-1} [16]. The result of FTIR of K indicates that amine showed the peak wavenumber at 1641,06 cm^{-1}, while BHA-GEL-K scaffolds at 1636,98cm^{-1}.The result from BHA-GEL-K scaffolds indicates that these scaffolds seems to contain marker from each component.

B. SEM-EDX

The result of SEM-EDX is shown in Fig. 3 and Table 1.The scaffolds contains carbon (C), oxygen (O), natrium (Na), magnesium (Mg), phosphor (P), and calcium (Ca). The order of the elements scaffolds is O> C> Ca> P> Na> Mg.

The spectrum of SEM-EDX shows that BHA-GEL-K scaffolds consist of C, P, O, Ca, Mg, and Na. C and O and it was obtained from basic structure of BHA, gelatin, and chitosan. Na was obtained from NaOH which is used to neutralize acetic acid in the preparation of scaffolds. BHA-GEL-K scaffolds contain 15,03 Wt% calcium and 9,22 Wt% phosphor. The ratio of Ca/P is 1,63. Ca and P is the major component of HA. Its standard ratio is at Ca/P 1,67 [4]. However, this study shows that the ratio of Ca/P is 1,63 and it makes this scaffolds contain a rich amount of Ca and P. Ca and P might support mineralization of bone and osteogenic differentiation.

C. Swelling studies

Swelling capability of scaffolds could facilitate cells in infiltrating into scaffolds. Its high capability of swelling would increase the surface area of the scaffolds very well. This might increase the probability of cell attachment to scaffolds. Swelling also plays an important role for the absorption of fluid from body, as well as the transfer of nutrients and metabolic waste. The higher WCP might increase the absorption of fluid from body so that the intake of nutrients to the cells in the scaffolds would increase.

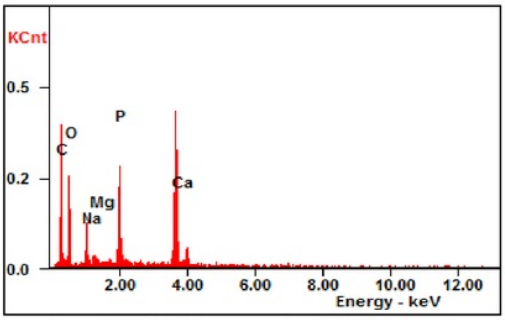

Figure 3. SEM-EDX spectrum of scaffolds BHA-GEL-K

Scaffold's swelling rate is at 1.8 to 2.5 and the WCP is at 65-71% [11]. However, BHA-GEL-K scaffolds seem to have *swelling* ratio at 3,00 ± 0,23 and the WCP at 74,90 ± 1,41%. It appears that this study has higher swelling ratio and WCP than previous research. This might happen because there is a difference of composition of gelatin and chitosan in scaffolds which have hydrophilic properties. The previous research used 2,5% organic component with gelatin and chitosan in 1:2 ratio, while in this study the author used 30% of organic component. The higher composition of chitosan and gelatin might increase its swelling ratio and WCP [4,17].

TABLE I. Table of element contain in scaffolds BHA-GEL-K

No.	Element	Wt%
1	C	35.11
2	O	35.30
3	Na	04.28
4	Mg	01.06
5	P	09.22
6	Ca	15.03

D. Degradation studies

Degradation rate plays an important role in the process of new bone formation [10]. Degradation rate might also be important for cell viabilities, cell growths, and host responses. Scaffolds should degrade and absorbed by body without leaving any trace. Degradation rate could be improved by polymer structures, properties, and environmental conditions.Degradation rate of scaffolds should be low because bone regenerations need a long time to complete its process [1]. Bone remodeling for human takes two to eight months to complete [18].

The degradation process in this study used distilled water that contains lysozyme. The N-acetyl glucosamine groups of chitosan chains could be hydrolyzed by lysozyme, while gelatin could be hydrolyzed with water. The degradation rate in gelatin and chitosan scaffolds should be high (around 40-50% after 7 days), however, in this study, it appears that the degradation rate is low (11,60%± 0,79) within 3 days. This happened due to difference of composition HA, previous research used 1wt% nano HA and this research used 70% BHA. An addition of BHA might reduce the accessibility of enzyme to the attacking site in polysaccharide molecule and it makes the degradation rate decreases [4].

IV. CONCLUSION

The scaffolds used were made of BHA, GEL, and K. FTIR study proves that BHA-GEL-K scaffolds consists of BHA, GEL, and K. SEM-EDX study shows that the scaffolds contains a rich amount of Ca/P that could support mineralization and osteogenic differentiation well. Based on the swelling study above, the result shows that the scaffolds might have good capability of absorbing fluid from body due to its swelling ratio and WCP above the standard and hydrophilic properties of its components which might increase probability of cell attachment, and have capability to absorb fluid that contain nutrient for bone formation process. The degradation study shows that BHA-GEL-K scaffolds has low degradation rate due to BHA in its components. After discovering this result, the author could conclude that BHA-GEL-K scaffolds might be a good candidate to support bone tissue engineering.

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PAGE 1

PAGE 2

PAGE 3

PAGE 4