Characterization and Degradation of Hydroxyapatite Gypsum Puger (HAGP) Freeze Dried Scaffold as a Graft Material for Preservation of the Alveolar Bone Socket

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Abstract

Hydroxyapatite Gypsum Puger (HAGP) used as a graft material is usually produced in the form of a porous scaffold that could serve of tissue engineering so graft materials have to be fully degradable. Biodegradation is essential as it allows for the space to be formed into bone and also blood vessel tissue could grow. The aim of this study is to analyze the characterization and degradation of HAGP freeze dried scaffold as a graft material for preservation of alveolar bone sockets.

The HAGP scaffold was made by mixing gelatin liquids using a sublimation/freeze dried system. Then, it was formed to a particle size of 150 to 355 µm. The structural properties and morphology of HAGP were characterized by X-Ray Diffraction (XRD) and Scanning Electron Microscope (SEM) and then compared with gold standard from Hydroxyapatite Bovine (HAB). The release of calcium (Ca) was tested using an in vitro degradation test-the AAS test and phosphorus release (P) was examined using a UV/Vis Spectrophotometer at days 1, 3, 7, 14 and 28.

The characterization of Freeze dried HAGP scaffold with XRD showed 100% of hydroxyapatite phase. The SEM photos showed many pores on the scaffold with an average size of 3 µm, therefore, the pattern was similar to the HAB scaffold (gold standard).

The degradation process of the Freeze dried HAGP scaffold slowly occurs which could affect the proliferation and the activity of the cells, thus it enters and grows into the scaffold to fabricate bone tissue.

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Introduction

Alveolar bone plays an important role in providing support for teeth. The loss of dental anatomy due to the extraction leads alveolar bone deficiency in the vertical and horizontal dimensions, and then progressive alveolar bone resorption will occur.[1-3] When the teeth are extracted, the trauma should be minimized and preservation of alveolar ridge sockets is required. The literature showed that early alveolar bone loss could be reduced significantly by giving grafting material to sockets.[1] Alveolar bone ridge protection has an effect on achieving optimal function in the results of prosthetic treatment.[3] Thus, the alveolar syringe preservation is required for bone replacement to restore the alveolar bone loss.[1]

This preservation is performed with graft materials.[3,4] One of the graph material that is being developed as a synthetic biomaterial is a hydroxyapatite bio ceramic with chemical formula of $Ca_{10}(PO_4)_6(OH)_2$. It has a hexagonal structure and a crystalline phase of the most stable of calcium phosphate compound.[5] The chemical composition of hydroxyapatite is similar to the

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inorganic component of human bone.[6] Hydroxyapatite also has a function to provide calcium and phosphate ions for bone regeneration process including osteointegration.[7] In previous research mentioned that hydroxyapatite could be synthesized from gypsum puger with similar characterization compared to 200 japan Hidrksiapatit as standard.[8] The production of hydroxyapatite gypsum puger (HAGP) is done by using freeze dried system.

Hydroxyapatite used as a graft material is usually produced in the form of a porous scaffold that could serve as a host for physiological biological activity of tissue engineering.[9,10] Implanted graft materials that have the right cellular affinity along with the potential for degradation are critical to the success of bone tissue engineering. Graft materials should have sufficient mechanical strength and also three-dimensional porous structure to provide bone remodeling.[11] These graft materials have to be fully degradable and the degradation is ideally suited to osteogenic levels.[12,13] Biodegradation is essential as it allows for the space to be formed into bone and also blood vessel tissue could grow. Biodegradation could be imagined as a process in which the materials decompose into simpler components, reducing the complexity of chemical compounds by the activity of cell biological systems, simple physical damage, and chemical erosion. Physical damage is usually due to the release and destruction of ions.[11,14]

This study used HAGP scaffold and HAB scaffold as a comparator, because Hydroxyapatite from Bovine was often used in the central installation bank of biomaterials of Dr. Soetomo General Hospital as graft materials for bone regeneration. The purpose of this study was to analyze the characterization and degradation of HAGP freeze dried scaffold as a graft material for preservation of alveolar ridge sockets.

Materials and methods

The Production of HAGP Scaffold

Gypsum powder from puger was sieved until it obtained <50 μm in particle size. Diammonium Hydrogen Phosphate (DHP) was weighed with mechanical scales to make a solution with a concentration of 0.5 M. The gypsum powder was weighed to be mixed with DHP solution which was 5 grams of powder and 400 mL DHP solution. Then, the solution was heated into microwave (hydrothermal process) at 100 °C for 30 minutes. Next, the solution was washed with aquades and filtered several times using filter paper until the pH was neutral. The powder was dried by microwave at 50 °C for 5 hours. Four grams of hydroxyapatite were mixed with gelatinous fluid. Solid gelatin was melted with hot water at a temperature of 60 °C to 10% liquid gelatin. Four grams of Hydroxyapatite was mixed with gelatin solution to obtain 10 ml of mixed liquid which was then frozen and dried with a freeze-dried system. Then, it was crushed, milled and sieved until it obtained 150-355 μm in particle size.

Characterization of HAGP Scaffolds

Compositional characterization was performed using XRD compared to the gold standard from HA Bovine, meanwhile for microstructural characterization was using SEM.

In Vitro Degradation Test of HAGP Scaffold

In vitro degradation test of HAGP scaffold was performed in artificial saliva that designed similar to the oral cavity. Scaffold HAGP+PEG (Poly Ethilene Glycol) was immersed in 10 ml of artificial saliva up to 28 days. Then, the release of calcium (Ca) was tested using an in vitro degradation test-the AAS test and phosphorus release (P) was examined using a UV/Vis Spectrophotometer at days 1, 3, 7, 14 and 28.[15]

Statistical Analysis

Normality test was performed using Kolmogorov Smirnov. Homogeneity test with Anova is used if the data distribution is normal; meanwhile Kruskal Wallis test is used if the data distribution is not normal.

Results

Characterization of HAGP Materials

The X-ray diffraction pattern (XRD) in the Hydroxyapatite Gypsum Puger (HAGP) was shown in Figure 1(a). There were three highest peaks in the XRD pattern on the HAGP scaffold that shown at d-spacing 3.42546 Å, 3.08845 Å, and 2.81286 Å. Also, figure 1(a) shows the three highest peaks on the HAB scaffold with the d-

spacing value of 2.79673 Å, 2.76307 Å, and 2.70481 Å. Figure 1(b) shows a similar combination of XRD pattern of HAGP and HAB scaffold.

The SEM image in Figure 2(a) shows a spherical HAGP scaffold of 150 to 355 µm and was porous with a size of about 3 µm. Figure 2 (b) shows a spherical HAB scaffold of 150 to 355 µm and was porous with a size of about 3.26 µm.

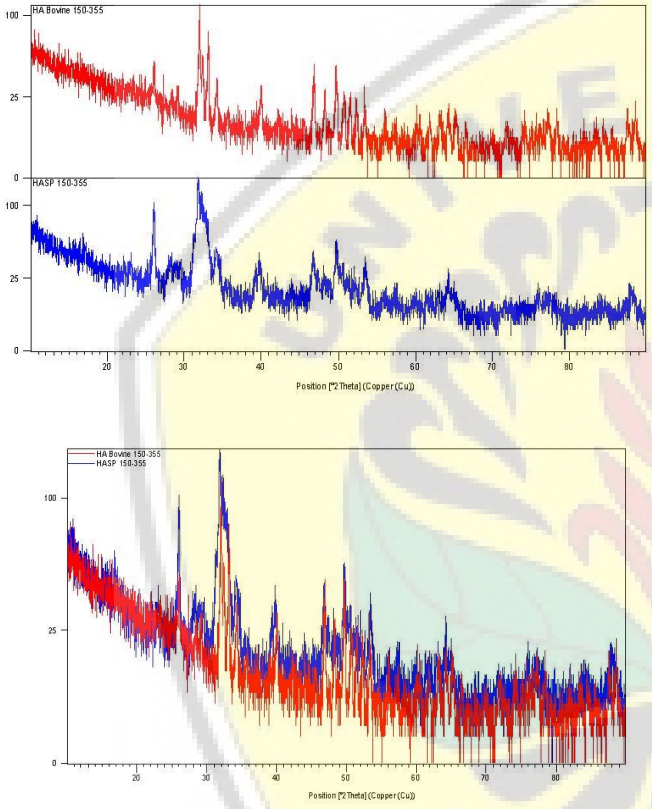

Figure 1. Materials characterization: (a) analysis of X-ray diffraction (XRD) on upperHydroxyapatite Gypsum Puger (HAGP) scaffold and lower Hydroxyapatite Bovine Scaffold (HAB), (b) Combined HAGP scaffold and HAB scaffold

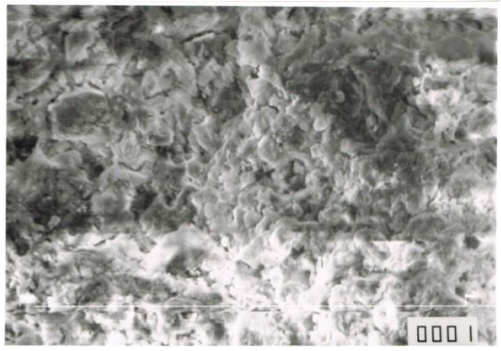

Figure 2. a

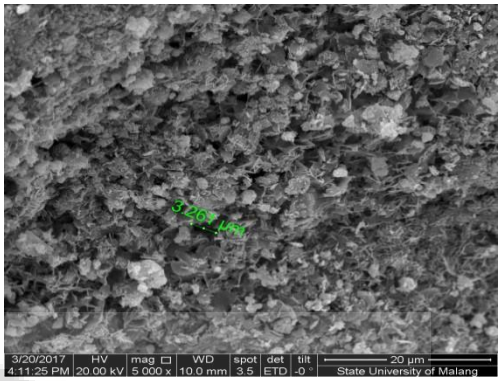

Figure 2. b

Figure 2. Scanning electron microscope (SEM): (a) on a Hydroxyapatite Gypsum Puger (HAGP) scaffold with 750x magnification, (b) Hydroxyapatite Bovine (HAB) Scaffold with 5000x magnification.

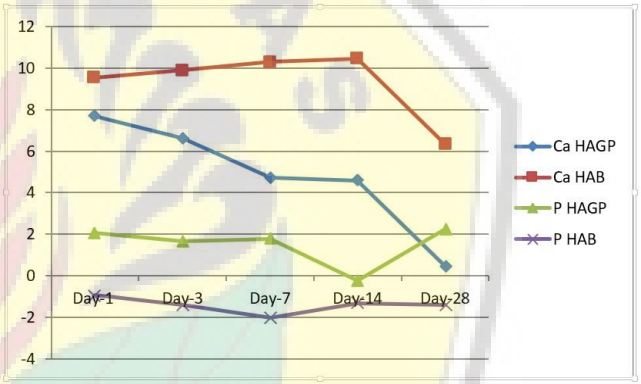

Figure 3. In vitro degradation of the release Ca and P ions on Hydroxyapatite Gypsum Puger (HAGP) scaffold and Hydroxyapatite Bovine (HAB) scaffold on days 1, 3, 7, 14, 28.

In Vitro Degradation Test of HAGP Scaffold

Ca ion concentrations in the HAGP and HAB scaffolds as the controls were measured using AAS. HAGP and HAB scaffolds were immersed in the artificial saliva for 28 days (Figure 3). Ca ion concentration of HAGP scaffold was high on the first day of immersion and then decreased on days 3, 7, 14 to day 28. There were significant differences between groups of day 1, 3, 7, 14, and 28. Ca ion concentration in HAB scaffold was slightly increased at day 14 but decreased again at day 28. There was no significant difference between groups of 1, 3, 7, 14, and 28. There was no significant difference between HAGP and HAB scaffolds on day 7, whereas there were

significant differences on days 1,3,14 and 28 (Table 1).

The release of P ions on the HAGP and HAB scaffolds was measured using the Spectrophotometer that shown in Figure 3. The release of P ions on the HAGP scaffold slightly decreased at day 14 but, statistically, there was no significant difference between groups of day 1, 3, 7, 14 and 28. The release of P ions on the HAB scaffold was statistically stable and there was no significant difference between groups of day 1, 3, 7, 14, and 28. There were no significant differences between HAGP and HAB scaffolds on days 3 and 14 whereas there were significant differences on days 1, 7 and 28 (Table 2).

Scaffold	Ca Release (x±SD)					*p*
	Day-1	Day-3	Day-7	Day-14	Day-28	
HAGP	7.70 ± 0.96[a]	6.63 ± 0.65[b]	4.73 ± 0.93[c]	4.60 ± 1.05[c]	0.46 ± 1.28[d]	0.000*
HAB	9.55 ± 0.36	9.91 ± 1.51	10.29 ± 3.73	10.45 ± 0.57	6.34 ± 2.80	0.168
p	0.012*	0.016*	0.083	0.000*	0.009*	

Table 1. Analysis of Ca release on in vitro degradation test. significant at $\alpha = 0.05$

Scaffold	P Release (x±SD)					P
	Day-1	Day-3	Day-7	Day-14	Day-28	
HAGP	2.05 ± 1.51	1.65 ± 2.26	1.79 ± 2.57	-0.22 ± 1.97	2.25 ± 1.66	0.436
HAB	-0.94 ± 1,.25	-1.39 ± 0.44	-2.01 ± 0.30	-1.32 ± 0.77	-1.42 ± 0.55	0.396
P	0.021*	0.072	0.026*	0.341	0.006*	

Table 2. Analysis of *p* release on in vitro degradation test.

Discussion

In this study, the samples used were HAGP scaffold with freeze dried system and HAB scaffold as the gold standard. The characterization analysis of HAGP scaffold composition and HAB scaffold obtained eight peaks on XRD pattern, this showed 100% of hydroxyapatite purity level, and XRD pattern on HAGP scaffold was similar to HAB scaffold as the gold standard. These results indicate that HAGP was successfully synthesized into HAGP scaffold.[8]

Microstructures characterization of HAGP and HAB scaffolds using SEM obtained many pores on a scaffold with an average size of 3 µm. This condition was highly conducive to cell activities, thus it enters and grows into the scaffold for bone remodeling and bone tissue engineering.[9-11, 17]

The degradation test could be shown by the release of Ca and P ions on the HAGP scaffold during the immersion on days 1, 3, 7, 14 and 28 in artificial saliva. The results of the study showed that the concentration of Ca ion release of HAGP scaffold at the beginning of immersion was high however; it was decreasing on day 28. It also occurred on HAB scaffold that there was a slight increase at the beginning of immersion then decreased on day 28. This means that there is a molecular chain breaking process when Ca concentration is increased and there is a precipitation process when Ca concentration decreases with high Ca concentration at the beginning of HAGP scaffold immersion in artificial saliva. These results were similar to the studies of Monteiro et.al. and Silva et. al.[15,18]

The release of P ions on the HAGP scaffold slightly decreased at day 14, whereas the HAB scaffold control group was more stable. However, statistically, there was no significant difference between the immersion groups of 1st, 3rd, 7th, 14th, and 28th day in the HAGP scaffold group and the HAB scaffold control group. This suggests that the decrease in release of P may be related to the phosphate product precipitation of hydroxyapatite in artificial salivary materials.[19,20]

The analysis using in vitro degradation test showed that both materials i.e., HAGP scaffold and HAB scaffold were degraded gradually. It was in accordance with previous researchers that the scaffold material should be slowly resorbed at some time until new bone forms to enter and grow with scaffold for bone tissue engineering.[21] The release of Ca and P from dissolved hydroxyapatite affects cell proliferation and metabolism.[20,22]

Conclusions

Characterization of compositions and microstructures of freeze dried HAGP scaffold by SRD test showed 100% hydroxyapatite purity levels and SEM obtained many pores on scaffolds with an average size of 3 µm. The results were identical or contained the same pattern with HAB scaffold (gold standard). The freeze dried HAGP scaffold degradation process

occurs slowly which can affect cell proliferation and cell activity, thus it enters and grows into a scaffold for bone tissue engineering. Therefore, the freeze dried Hydroxyapatite Gypsum Puger (HAGP) scaffold is one of the most usable graft materials as an alternative material for the preservation of alveolar bone sockets.

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Declaration of Interest

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