Study on Biocompatibility of Chitosan/Hydroxyapatite Doped Silicon Composite as Material for Alveolar Socket Preservation

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Abstract. As the Alveolar Socket Preservation (ASP) was developed as a procedure for placement of bone graft on sockets where the tooth was removed, a new composite of Chitosan/Hydroxyapatite doped Silicon (Chi/HAp-Si) was synthesized. Si will be added to HAp in amount 7 wt% by a solid-state method. As a comparison, the HAp-Si later will be mixed with chitosan with variations of 0, 5, 10, and 15 wt%, with the aim of enhancing antibacterial ability against S. aureus bacteria. The synthesized samples were characterized using XRD and SEM. Antibacterial activities of the Chi/HAp-Si composite were evaluated against S. aureus bacteria. The addition of chitosan did not have much effect on the biocompatibility of HAp-Si, especially in S. aureus bacteria. However, the addition of chitosan to the 10 wt% composition has the highest antibacterial properties.

1. Introduction
Tooth extraction is one of the curative measures performed by dental practitioners that aims to remove teeth from the alveolar bone socket [1]. The alveolar bone is a type of bone that is designed to accommodate the teeth, found in the upper jaw and the lower jaw. The shape and volume of alveolar bone are determined by the presence or absence of teeth, tooth shape, and the direction and axis of tooth extraction. After tooth extraction is done, the process of physiological re-formation of hard and soft tissue will result in dimensional depreciation. Reduction of alveolar volume will reduce aesthetics as well as harm functional bones, and complicate the installation of implanted installations [2]. Therefore, preservation of the alveolar socket (ASP) was developed to maintain the volume of alveolar bone that existed after tooth extraction at once to ensure adequate bone profile support.

Hydroxyapatite (HAp) is one of the most common inorganic materials used as the bone graft for ASP procedure [3], [4]. However, HAp is a material that is only osteoconductive, but not osteoinductive [5]. This makes the effectiveness of HAp as a low bone graft. Therefore, the modification of HAp with the addition of silicon as ionic doping is done as a way to conquer the weakness of HAp. Silicon is investigated capable of promoting the transformation of the surface of the material into an apatite that is biologically equivalent. Silicon is considered to be an element that plays an important role in the manufacture of composite bone grafts [6], [7].

Because of its use in the human body environment, the biocompatibility of material is considered. Some bacteria that often appear in orthopedic dental are anaerobic bacteria, which is a type of bacteria...
that can live with or without oxygen. The most common bacterial samples are Staphylococcus aureus [8], [9]. Therefore, biocompatibility testing was performed by an antibacterial test against S. aureus bacteria with a diffusion disk method. As a comparison, the HAp-Si later will be mixed with chitosan with variations of 0, 5, 10, and 15 wt%, with the aim of enhancing antibacterial ability toward S. aureus bacteria. Characterization was performed by XRD testing for analyzing the HAp-Si phase conducted and its effect on the XRD pattern because of the chitosan addition, and SEM testing for comparison of morphological analysis between the HAp-Si and K/HAp-Si composite.

2. Experimental Method

2.1. Materials
HAp powder (Ca$_{10}$(PO$_4$)$_6$(OH)$_2$) was purchased from PT Rekan Kita Bersama, and silica powder (SiO$_2$, purity $\geq$ 99.5% ACS Grade) was purchased from PT. Solechan MT with CAS number 7631-86-9. The Chitosan was purchased from CV. Bio Chitosan Indonesia, with particle size 100 mesh.

2.2. Synthesis of Hap-Si
In this study, the HAp doped Si was prepared by a solid-state method using HAp powder and SiO$_2$ powder. The SiO$_2$ was measured 7 wt% HAp. Both of HAp and SiO$_2$ later grounded using mortar and pestle to homogenized the particle, and then put into a crucible and then sintered for 20 h at 1100°C.

2.3. Synthesis of K/HAp-Si
The synthesis was begun by dissolving chitosan powder with 100 ml of 3% acetic acid, stirring for 1 hour with constant speed at 70 °C. The addition of chitosan (K) was varied 0, 5, 10, 15 wt% of HAp-Si. The chitosan solution later was added to 10 ml of distilled water that already preheated to 70°C, before mixed by the HAp-Si. The pouring of the HAp-Si into the solution is carried slowly, then the solution is again stirred at 70 °C with the same rate of speed until the result is a thickened slurry. The slurry was then sterilized for approximately 24 hours, then dried oven at 70 °C for more than overnight.

2.4. Characterization of HAp/Si and K/HAp-Si
The sample characterization equipment was principally used for three types of analysis. Phase analysis was performed using X-ray diffractometer (XRD) Philips at Laboratorium of Materials Characterization ITS, Surabaya, surface microstructure analysis observed with Scanning Electron Microscope (SEM) FEI type INSPECT S50 at Laboratorium of Materials Characterization ITS, Surabaya, and antibacterial analysis using diffusion disk method at Biology Laboratory of ITS, Surabaya.

3. Results and Discussion
The doping of Si to HAp would substitute the phosphate group on the hydroxyapatite. Some of the OH-groups probably would be lost to retain charge balance, to compensate for the extra negative charge of the silicate groups accordingly. The reaction is given in (1):

$$\text{Ca}_{10}$(PO$_4$)$_6$(OH)$_2$ + xSiO$_2$ $\rightarrow$ Ca$_{10-x}$(PO$_4$)$_6-x$(SiO$_2$)$_x$(OH)$_2$ + xPO$_3^-$ + OH$^-$ (1)

3.1. XRD Results
Figure 1 shows a comparison between each variation. After matched with JCPDS 00-034-0010, the pattern indicates the phase HAp successfully conducted, and no peak indicates pure Si. Similar with previous study, adding element such as Mg into K/Hap obtained no individual peak of crystalline Mg detected in the XRD pattern. It indicates that element particles such as Mg or Si have been micro joined with hydroxyapatite [10]. Based on Figure 1, it can be seen that along with the addition of chitosan, there is a decrease in intensity and peak shift HAp-Si which indicates the occurrence of the bond between HAp-Si with chitosan by a composite process [11]. This occurs because the structure of chitosan is more amorphous than HAp, so when combined it will change the XRD pattern. However, unidentified new peaks indicate no chemical bonding between chitosan and HAp-Si. The bonds that occur are physical bonds so that the peak characteristics of chitosan and HAp-Si appear individually on XRD results.
Figure 1. Comparison of XRD test results graph with HAp-Si variation, 5% K/HAp-Si, 10% K/HAp-Si, and 15% K/HAp-Si

3.2. Antibacterial Testing Results
Based on Table 1, it can be seen that the addition of chitosan composition enhances the antibacterial ability of the composite sample, indicated by the addition of inhibition diameter (shown by the clear zone appeared around the sample; Figure 2), but decreases when the addition exceeds 10 wt%. This probably because of the high concentration of chitosan will produce a thick solution [12]. Furthermore, a solution that is too thick will be difficult to make diffusion to HAp-Si, compared with a more dilute solution. As a result, the data indicates a decrease in inhibitory activity, with the shrinking diameter of the clear zone.

Table 1. Measurement of the inhibition diameter

| Samples          | Inhibition diameter (mm) |
|------------------|--------------------------|
| A (HAp-Si)       | 0.93                     |
| B (5%K/HAp-Si)   | 0.47                     |
| C (10%K/HAp-Si)  | 0.50                     |
| D (15%K/HAp-Si)  | 0.37                     |

Figure 2. Visual observation of antibacterial activity testing of S. aureus bacteria for 12 Samples with different variations (A1-A3 → HAp-Si, B1-B3 → 5%K/HAp-Si, C1-C3 → 10%K/HAp-Si, D1-D3 → 15% K/HAp-Si)
From the data in Table 1, it can also be seen that the clear zone diameter of HAp-Si without the addition of chitosan has a higher value. This suggests that S. aureus bacteria are more susceptible to HAp-Si, and the addition of chitosan does not significantly affect antibacterial activity against S. aureus. The comparison of the results obtained was reinforced by the SEM test results, where the addition of Si and chitosan reactions have different reactions to the HAp microstructure.

3.3. SEM Results
After performing XRD and antibacterial testing, it is known that as composites, the composition of 10 wt% of chitosan is the best material in this study for the preservation of alveolar sockets. The composition of 15 wt% chitosan is the material with the least antibacterial ability. Based on the results of antibacterial tests also, it was found that the sample with a composition of 0 wt% chitosan (100% HAp-Si) actually has a higher antibacterial value. The comparison of these results is then corroborated by observation of surface morphology using SEM. The result of the SEM HAp-Si test with 100x magnification can be seen in Figure 3.

![SEM test results](image)

**Figure 3.** SEM test results with magnification of 10,000x (a) HAp-Si, (b) 5% K / HAp-Si, (c) 10% K / HAp-Si, and (d) 15% K / HAp-Si

From Figure 3, it can be seen that the addition of Si decreases the porosity of HAp thus the microstructure gained more fused among the particles to form clumps of the coating layer. This result is in accordance with previous research [13]. The decrease in porosity reveals that the incorporation of silicon into HAp occurs in the HAp pore system. This coupling can then help dissolve the surface ions that stimulate the formation of the apatite layer [14]. The addition of chitosan to HAp-Si also not only alters the XRD HAp-Si pattern, but also alters the sample morphological structure by producing increasingly coarse and irregular surfaces [15].

After the addition of chitosan, there is interconnected tissue between HAp-Si, turning the microstructure into spherical chunks. When the composition of chitosan is low, chitosan is unable to envelop the HAp-Si particles, so that chitosan will only become the granular adhesive (shown in Figure 3 (b)). The increased chitosan composition to 10 wt% indicates enough chitosan composition to be a connecting tissue so that HAp-Si increasingly conducts spherical chunks and increases porosity between chunks (shown in Figure 3 (c)). The increasing chitosan composition further reinforces the shape of HAp-Si and makes porosity between HAp-Si getting larger [16].

Based on SEM results it can be seen the correlation between the antibacterial properties with microstructure formed. The surface of the HAp-Si microstructure without the addition of chitosan has a
small porosity so that complete inhibition of the surface can occur optimally through the reaction between samples with bacterial incubation, compared with HAp-Si mixed with chitosan. Therefore, the three results of the sample characterization test indicate that the addition of chitosan does not have much effect on the biocompatibility of HAp-Si, especially in S. aureus bacteria.

4. Conclusions
The addition of chitosan (K) does not have much the biocompatibility of HAp-Si, especially in S. aureus bacteria. However, the addition of chitosan to the composition of 10 wt% is the optimal composition in terms of antibacterial activity and the probability of the apatite layer formed from the composite surface. The XRD intensity decreases along with the addition of chitosan. While the SEM morphology structure of the samples producing more increased coarse and irregular surfaces with the addition of chitosan to Hap-Si. Material with this composite composition can be suggested to be the material for Alveolar Socket Preservation (ASP).

5. References
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