Abstract

Background. Bone loss rapidly increases 6 months post tooth extraction, which causes the atrophy of the alveolar bone. Two kinds of biomaterials which can stimulate bone regeneration are bioceramics and polymers. Making a composite of biomaterials results in better physical and biomolecular characteristics in comparison with a bioceramic or a polymer alone. Hydroxyapatite nanoparticles (HANPs) are one of the bioceramics commonly used for bone regeneration; they can degrade faster than hydroxyapatite (HA) microparticles, but have an insufficient pore size. Polyvinyl alcohol (PVA) and poly lactic-co-glycolic acid (PLGA) are polymers which have been used for biomedical applications. However, PLGA alone has insufficient cell attachment and PVA alone slowly degrades in the bone tissue.

Objectives. The aim of the present study was to analyze the biodegradation properties of the HANP/PLGA/PVA composites and investigate the pore size.

Material and methods. The HANP/PLGA/PVA composites were prepared using the freeze-drying method, with 20% (w/w) of HANP and 20% (w/w) of PLGA. Morphology and the pore size were determined by means of the field emission scanning electron microscopy (FE-SEM) analysis. Biodegradation properties were determined by calculating water uptake and water loss for 1, 3 and 6 weeks. Statistical analysis was performed based on the one-way analysis of variance (ANOVA) at \( p < 0.05 \).

Results. The HANP/PLGA/PVA composites had the greatest mean pore size and a rougher surface than others (176.00 ± 61.93 μm; \( p < 0.05 \)). Moreover, the HANP/PLGA/PVA composites had the greatest water uptake, significantly in the 3rd (730.46%; \( p < 0.05 \)) and 6th weeks (731.07%; \( p < 0.05 \)), and water loss in the 6th week (67.69%; \( p < 0.05 \)).

Conclusions. The HANP/PLGA/PVA composites have optimal pore size, morphology and degradability, which shows their high potential as an effective bone scaffold to repair the alveolar defect post tooth extraction.

Key words: biodegradable, bone regeneration, hydroxyapatite nanoparticle, polyvinyl alcohol, poly lactic-co-glycolic acid
Introduction

Tooth extraction is one of the most common kinds of dental treatment in developing countries. A total of 944 tooth extraction procedures were performed in 450 patients throughout the year 2014 at Jember Dental Hospital, Indonesia, indicating that each patient was subjected to tooth extraction treatment at least twice a year, on average. Tooth extraction may negatively impact the alveolar bone, which can result in the atrophy of the alveolar bone, the collapse of the soft tissue, and a short and narrow alveolar ridge. Other consequences of bone loss include the reduction of esthetics, the inhibition of mastication processes, and the insufficient support of dental implants or prosthetic restorations. Accordingly, it is important to develop biomaterials which could stimulate bone regeneration and prevent bone loss, or for alveolar preservation.

Bone is an inorganic-organic composite material consisting of hydroxyapatite (HA) as the main component. In Indonesia, the most widely used bone grafting material to avoid bone loss following tooth extraction is HA. However, HA slowly degrades, over approx. 24 months. It is a brittle material, and thus its application is limited to low-pressure areas. Hydroxyapatite nanoparticles (HANPs) are one of the bioceramics that have better osteoconductivity, biocompatibility and biodegradability, and also exhibit enhanced osteoblast adhesion as compared to those of conventional HA. This bioceramic may also be able to increase the tensile strength of the scaffold. However, HANPs have insufficient porosity and pore size. The ideal range of the pore size to promote bone regeneration is 50–300 µm. Accordingly, composites constituted by a combination of HANPs and other materials are needed to overcome the limitations of conventional HA.

Polyvinyl alcohol (PVA) and poly lactic-co-glycolic acid (PLGA) are synthetic polymers which possess good stability for bone repair and regeneration. Polyvinyl alcohol exhibits highly favorable properties, such as biocompatibility, and physicochemical characteristics, and has been used for biomedical applications. It has shown better mechanical stability than other polymers, as demonstrated in previous studies. However, the degradation of PVA is very slow. Poly lactic-co-glycolic acid is one of the best biodegradable materials, which is also used as a drug carrier. It degrades into non-toxic products. Nevertheless, neither PVA nor PLGA support cell adhesion. They also have low mechanical support. One study showed that the addition of HA to a polymer scaffold resulted in enhanced osteoblast attachment, mineralization and metabolic activity.

The present study focused on the development of the HANP/PLGA/PVA composites by using the freeze-drying method. This study aimed to analyze the biodegradation properties and pore size of the HANP/PLGA/PVA composites. The findings of the study are expected to provide suggestions for synthetic bone grafts to avoid or recover bone loss following tooth extraction.

Material and methods

The following components were used: HANPs ±60 nm (BATAN, Jakarta, Indonesia); PLGA 50:50 Mw 15,000–25,000 Da (PolySciTech, West Lafayette, USA); PVA fully hydrolyzed Mw 73,000 Da (Merck, Kenilworth, USA); phosphate-buffered saline (PBS) (Gibco™, Thermo Fisher Scientific, Waltham, USA); and ethyl acetate (Merck). This study was conducted in May 2019. The study materials were divided into 4 groups: G1 (PVA alone); G2 (HANP/PLGA); G3 (PLGA/PVA); and G4 (HANP/PLGA/PVA). The HANP/PLGA/PVA composites contained 20% (w/w) of HANP and 20% (w/w) of PLGA. All of these composite specimens were prepared by means of the freeze-drying method at −80°C for 24 h.

The pore size analysis was determined by field emission scanning electron microscopy (FE-SEM) (FEI Quanta™ FEG 650; Thermo Fisher Scientific, Waltham, USA).

The biodegradation test was performed in triplicate (n = 3) by immersing the composites in 10 mL of the PBS solution (pH 7.4). They were then incubated at 37°C for 1, 3 and 6 weeks. All the composite specimens were weighed before immersing to determine their initial weight (W_i). At the end of each degradation period, the swollen weight was measured immediately (W_s). The composite specimens were then dried to determine the final dried weight (W_f). Subsequently, the water uptake and water loss values were also determined. To calculate the water uptake value, equation 1 was used:

\[
\text{Water uptake} = \left( \frac{W_s - W_i}{W_i} \right) \times 100 \% \quad (1)
\]

where:
- W_s – swollen weight [mg];
- W_i – final dried weight [mg].

Additionally, the water loss value was calculated with equation 2:

\[
\text{Water loss} = \left( \frac{W_i - W_f}{W_i} \right) \times 100 \% \quad (2)
\]

where:
- W_i – initial weight [mg];
- W_f – final dried weight [mg].

Each measured sample contained 3 parallel test samples. Statistical analysis was performed using the one-way analysis of variance (ANOVA) (IBM SPSS Statistics for Windows, v. 25.0; IBM, Corp., Armonk, USA) and was considered statistically significant at p < 0.05.
Results

Figure 1 shows the FE-SEM images of the surface morphology and pore size of the composites. The representatives of the FE-SEM images show the natural distribution of the pore formation in the scaffold. Table 1 shows that the HANP/PLGA/PVA composites had the greatest pore size. Table 2 presents the initial weights, swollen weights and final dried weights of all composite specimens, which influenced the percentage of water uptake and water loss. Figure 2 shows the comparison of water uptake percentages for all composite specimens at different periods of time. The HANP/PLGA/PVA composites showed the highest water uptake percentage in the 3rd and 6th weeks (730.46% and 731.07%, respectively), indicating that water absorption was increased 7-fold as compared to the initial weight. Figure 3 shows that the HANP/PLGA/PVA composites exhibited a greater water loss value in the 6th week (67.69%) than other composites. With the absence of PLGA, the swelling ratio (water uptake percentage) of the HANP/PVA composites was half that of the HANP/PLGA/PVA composites, indicating that the incorporation of PLGA into the scaffold enhanced the biodegradation rate of the composites.

Table 1. Pore size of the composites ($n=3$)

| Composite           | Pore size [µm] |
|---------------------|----------------|
| PVA alone           | $51.34 ±31.85$ |
| HANP/PVA            | $54.53 ±35.74$ |
| PLGA/PVA            | $138.46 ±68.54^*$ |
| HANP/PLGA/PVA       | $176.00 ±61.93^*$ |

Data presented as mean (M) ± standard deviation (SD).

* statistically significant differences in relation to all other groups ($p < 0.05$).

Fig. 1. Morphological analysis of the composites with field emission scanning electron microscopy (FE-SEM)
A – PVA alone; B – HANP/PVA; C – PLGA/PVA; D – HANP/PLGA/PVA.
PVA – polyvinyl alcohol, HANP – hydroxyapatite nanoparticle, PLGA – poly lactic-co-glycolic acid.

Fig. 2. Percentages of water uptake of the composites
*p < 0.05.
Discussion

Over the last decade, polymer and bioceramic composites have attracted much attention as biomaterials to be developed for bone regeneration.12,14,24,25 Figure 1 shows that the surface area of the HANP/PLGA/PVA composites was rougher than that of other composites, indicating that the addition of HANPs to the scaffold increases the surface area and its roughness.15 The surface roughness of composites influences their interaction with the biological environment.26,27 It also enhances cell adhesion, differentiation and proliferation.26–29 The pore size is mainly responsible for cell proliferation, migration and nutrition.15,16 Specific cells require different pore sizes for optimal attachment and proliferation.26 Research by Chang and Wang showed that osteoblast migration was faster through scaffolds with a pore size larger than 100 µm.26 A study by Loh and Choong demonstrated that the optimal pore size of a scaffold to enhance cell migration and proliferation ranged from 100 µm to 350 µm.15 An interconnected network serves the improvement in the mechanical stability of the implant through the incorporation of PLGA.30 Moreover, the selection of the technique to prepare the composite scaffold for bone regeneration has a great impact on the pore size and porosity of the scaffold.15,16,31,32 Freeze drying is a conventional technique that allows frozen water to sublime directly and results in pore formation.15,31,32

The presence of HANPs in the composites could affect the surface roughness and increase the surface area. The incorporation of PLGA into the scaffold could increase the pore size and biodegradation rate of the composites as compared to those of PVA alone or HANP/PVA. The HANP/PLGA/PVA composites demonstrated optimal pore size, morphology and degradability, which indicates their high potential as an effective bone scaffold to repair the alveolar defect following tooth extraction or for alveolar preservation.

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Conclusions
The presence of HANPs in the composites could affect the surface roughness and increase the surface area. The incorporation of PLGA into the scaffold could increase the pore size and biodegradation rate of the composites as compared to those of PVA alone or HANP/PVA. The HANP/PLGA/PVA composites demonstrated optimal pore size, morphology and degradability, which indicates their high potential as an effective bone scaffold to repair the alveolar defect following tooth extraction or for alveolar preservation.

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Table 2: Weights of the composites (n = 3)

| Composite          | W_i (mg) | W_s (mg) | W_f (mg) |
|--------------------|----------|----------|----------|
| PVA alone          | 340.00 ±12.29 | 768.00 ±28.16 | 180.33 ±15.70 |
| HANP/PVA           | 401.30 ±64.53 | 894.67 ±66.66 | 177.00 ±4.00 |
| PLGA/PVA           | 352.67 ±95.70 | 913.00 ±75.5* | 179.67 ±11.15 |
| HANP/PLGA/PVA      | 348.67 ±49.89 | 902.33 ±16.26 | 200.33 ±18.77 |

Data presented as M ±SD.
W_i – initial weight; W_s – swollen weight; W_f – final dried weight; * statistically significant differences in relation to all other groups (p < 0.05).
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