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Novel biodegradable hydrogel scaffold based on hydroxyapatite eggshell, collagen, and epigallocatechin-3-gallate

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ABSTRACT

Background: Biodegradable hydrogel scaffold is one of the crucial characteristics that determine the success of pulp regeneration. The degradation should be suitable for the growth of new tissue establishment. The aim of this study is to synthesize and compare the novel biodegradable hydrogel scaffold based on hydroxyapatite (HAp) eggshell, collagen, and epigallocatechin-3-gallate (HAp-Col-EGCG) with different HAp concentrations *in vitro*.

Materials and Methods: This study is original research. HAp-Col-EGCG hydrogel scaffolds were prepared using 1:1, 1:2, and 1:4 ratios of collagen and HAp with 10 μmol/L EGCG. The samples were freeze-dried and immersed in phosphate buffer saline containing lysozyme enzyme. The dried samples were weighed to determine the percentage of biodegradation value ($P < 0.05$).

Results: The result showed HAp-Col-EGCG was biodegradable but it has not been concluded that it can be completely eliminated. The data were analyzed by one-way analysis of variance and it indicated significant differences in percentage values.

Conclusion: Hydrogel scaffold based on HAp-Col-EGCG can be degraded and have the potential to be used as a biodegradable scaffold in supporting tissue regeneration.

Key Words: Biodegradable, collagen, eggshell, epigallocatechin-3-gallate, hydrogel, hydroxyapatite, scaffold

INTRODUCTION

Hydroxyapatite (HAp) is one of the main mineral and bone components with the formula $Ca_{10}(PO_4)_6(OH)_2$. HAp is a calcium phosphate compound and is often used as a biomaterial because of its biocompatible, nontoxic, and bioactive characteristics. It can also degrade and adsorb on molecular bioactive surfaces.[1] Because of these beneficial characteristics, HAp can be very useful in tissue engineering or other medical applications. The limitation of HAp is its fragile property. According to several studies, making a composite material is recommended.[2]

HAp can be synthesized from natural sources such as animal bones, corals, eggshells, and more.[3,4] Natural sources of HAp have more apatite crystals compared to synthetic sources.[5] Eggshells contain calcium carbonate that can be synthesized into calcium oxide (CaO). CaO will transform into calcium hydroxide and it will go through a calcination process until HAp powder is formed.[6] Eggshell HAp

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forms various porous scaffolds that can support cell regeneration and has osteoconductive properties. Hence, nowadays, it is popularly used.[1] It can form into a hydrogel scaffold with low viscosity to support the delivery of biological molecules and be injected into certain areas.[7,8]

Meanwhile, collagen is a fibrous protein that is found in vertebrae extracellular matrix. Usually, collagen is used to form films, foam, fiber, and ligaments in biomedical applications. Collagen scaffold has a high porosity and good permeability.[9] It can guide tissue regeneration and provide physical support to networks.[9-11] The composite material between HAp and collagen can form mineralized collagen.[10]

Furthermore, epigallocatechin-3-gallate (EGCG) has pleiotropic effects such as antioxidant, anti-inflammatory, and neuroprotective effects. It can protect tissue from damage.[12] It can strengthen the collagen structure and enhance its properties. It can also enhance osteoblast proliferation and differentiation.[10] To increase the benefit of each material, HAp, collagen, and EGCG, the composite polymer was synthesized as a hydrogel scaffold using the sol–gel method.

As an ideal scaffold, HAp-Col-EGCG hydrogel must have several characterizations. One of them is biodegradation. Biodegradation means the scaffold should resorb through natural processes or the elimination of polymers due to cellular activity.[13] The biodegradation process should harmonize with tissue remodeling and support organizing intercellular space so that it can be replaced by new tissue.[14] The purpose of the present study was to evaluate the biodegradation of HAp-Col-EGCG hydrogel scaffold with different ratios of HAp in 3,7, and 14 days.

MATERIALS AND METHODS

The formulation of hydroxyapatite, collagen, and epigallocatechin-3-gallate hydrogel scaffold

This study is original research. HAp was obtained from eggshell (Pro-dbLC, Pertiwi Technology, Bogor Indonesia). Collagen bovine type I (Gibco, Thermo Fisher Scientific, USA), EGCG (Sigma-Aldrich NoE4268 EGCG ≥80%, USA), HMPC 2% (Benecel K100M, Ashland, USA), sodium hydroxide 1 N (Merck, USA), phosphate buffer saline (PBS) (Gibco, Thermo Fisher Scientific, USA), deionized water, and sterile distilled water (Merck Millipore Milli-Q) were also used.

The synthesis of HAp-Col-EGCG hydrogel scaffold was initiated by dissolving 1%, 2%, and 4% HAp with deionized water in magnetic stirrers for 1 h at 350 rpm.[15] Subsequently, 10 µMol/L EGCG was added to each solution and stirred until homogeneous.[16] Furthermore, collagen was prepared at a 3 mg/ml concentration and mixed with PBS, sodium hydroxide, and distilled water.[17] Collagen solution, HAp, and EGCG solution were stirred together for 30 min in the magnetic stirrer. The final component, HPMC was then added to the formula to make a gel form.[18,19]

The biodegradation value measurement

The HAp-Col-EGCG hydrogel scaffold was frozen at −40°C for 2 h and freeze-dried for 24 h.[20] The measurement of hydrogel scaffold biodegradation was initiated by weighing the dry scaffold first. Then, it was immersed in PBS with a 1.6 µg/mL concentration of lysozyme enzyme.[21] The concentration of the lysozyme enzyme was similar to the enzyme content in human serum. The PBS solution was changed every day to maintain the effectiveness of the lysozyme enzyme. On the 3rd, 7th, and 14th days, the samples were removed from the solution. They were washed with distilled water, and later freeze-dried. The dried scaffold was weighed, and the biodegradation percentage was calculated by this formula:[22-24]

$$\text{Degradation rate } (\%) = \frac{(\text{Wo} - \text{Wt})}{\text{Wt}} \times 100\%$$

Wo: Initial weight

Wt: Weight in t days

Statistical analysis

The quantitative data were described as mean and standard deviations. The data normality was examined using the Shapiro–Wilk test. The differences within the same groups were analyzed using the GLM repeated measure one-way analysis of variance (ANOVA), and the comparation between groups were evaluated using one-way ANOVA, followed by the *post hoc* Tukey test, using SPSS statistic software (SPSS Inc., Chicago, Illinois) ($P < 0.05$).

The limitations of this study are that the biodegradation was measured only at certain times (3, 7, and 14 days). The immersion time could be extended according to the ideal time to provide network remodeling opportunities. Other factors to support the potential uses of HAp-Col-EGCG

hydrogel scaffold in tissue engineering have also not been researched in this study.

RESULTS

As shown in Figure 1, in the same groups of 1% HAp concentration, there were no differences in the biodegradation values on the 3rd and 7th days, and the 3rd and 14th days. However, there was a significant difference on the 7th and 14th days of observation ($P < 0.05$). Within the 2% and 4% HAp concentration groups, there were differences found on days 3rd and 14th and also on days 7th and 14th.

Furthermore, the comparation among the groups showed there were no significant differences in the 1%, 2%, and 4% HAp concentration groups on days 3rd and 7th. However, there were significant differences on day 14th between the 1% and 4%, and also the 2% and 4% HAp groups. The data are shown in graphs [Figure 1]. In this study, the HAp-Col-EGCG hydrogel scaffold has proven to be biodegradable. More HAp concentration performed less biodegradability in all samples.

DISCUSSION

This study has proven that all groups of scaffolds were biodegradable in PBS solution containing lysozyme enzyme that is akin to the human serum condition.[22] Usually, the polymer degradation process occurs because of simple dissolution, hydrolysis, oxidation, and enzymatic activity. The hydrolytic process is related to the breakdown of covalent bonds with water.[13,25] The degradation process will reduce the polymer molecular weight.[26] The

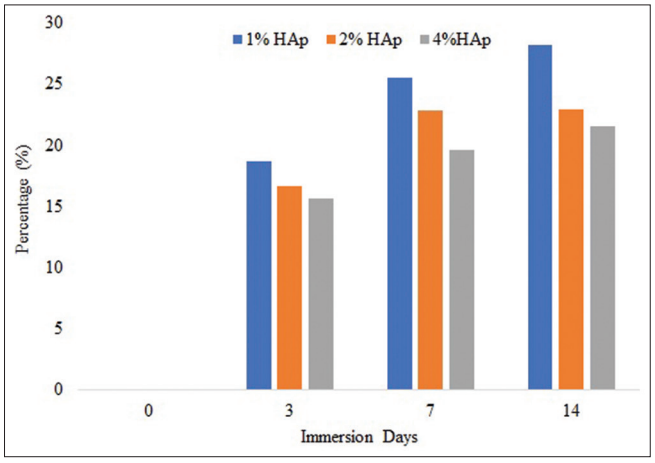

Figure 1: The degradation value of HAp-Col-EGCG Scaffold on the 3rd, 7th, and 14th days

enzyme degradation occurs because the peptide bond cleavage process becomes a hydrogel polymer chain.[25] Proline side chain in collagen is known to be related to collagen triple helix stability.[27] In the latest study, EGCG and collagen have a hydrogen bond, and EGCG could stabilize the active component. Its chemical structure could support biological collagen characterization.[28]

Some factors that affect the level of degradation are the structure and molecular weight of the material. The higher the molecular weight, the harder it is to degrade. The scaffold structure that also affects the biodegradation process is the surface ratio to volume and the diameter of porosity. Time is also an essential factor to be considered.[26] Ideal scaffold degradation should be compatible with the values of new tissue growth. Too fast degradation will reduce the scaffolding ability of the cell supporting factor, and too slow degradation will inhibit the formation of new tissue.[25]

The HAp-Col-EGCG composite scaffold has proven to be gradually biodegradable in weight in certain incubation times. The reduction of HAp content in composite scaffolds has substantial biodegradation.[29] In the present study, the higher HAp concentration (4%) showed a decrease in biodegradation ability. This supports the latest study that higher HAp is related to a low rate of biodegradation value.[1] It also proved the existence of interaction among collagen, EGCG, and HAp. In the current study, time also affected the degradation. The weight loss of scaffold with 1%, 2%, and 4% HAp concentrations on the 3rd day was between 15.66% ± 9.39%, 16.69% ± 2.03%, and 18.72% ± 4.01%. On the 7th day, the scaffold degradation was higher compared to the incubation time; they were 19.61% ± 4.90%, 22.82% ± 5.47%, and 25.48% ± 7.08%. Next, on the 14th day, the biodegradation occurred at 28, 19% ± 2.23%; 22.9% ± 0.96%; 21.58% ± 2.11% of its original weight. We found that the rate value of biodegradation was higher on days 3–7, and lower on days 7–14 days.

The degradation of the HAp-Col-EGCG hydrogel scaffold is suspected to be related to HAp, collagen, EGCG composition, incubation time (day), and physiological temperature. There is also correlated to the lysozyme or enzymatic solution because the collagen is included in an easily biodegradable natural polymer. The most weight loss was noticed

in the less HAp concentration among all the groups. This supports previous studies that observed the biodegradation of microsphere HAp and magnesium phosphate composite scaffold.[29,30] We also found that the biodegradation time can be enhanced by intensifying the HAp concentration. All the samples degraded continuously and the percentage was about 4%–15% weekly. Several factors have to be analyzed to support the HAp-Col-EGCG scaffold potential in pulp regeneration, such as its biocompatibility, swelling ratio, and other issues before clinical application.

CONCLUSION

This study discovered that HAp-Col-EGCG hydrogel scaffold can be degraded. However, biodegradation occurs gradually over time, and a higher HAp concentration is resistant to degradation. This scaffold could be riven by lysosomal enzymes. Further observation of HAp-Col-EGCG hydrogel scaffold characterization should be done to confirm its potential use in tissue regeneration fields.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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