ORIGINAL ARTICLE

Biodegrable Collagen, Hydroxyapatite, and Epigallocatechin-3-Gallate Hydrogel Scaffold as an Induction Material for Pulp Dentin Regenaration

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ABSTRACT

Introduction: Current regenerative endodontic treatment approaches preserve pulp vitality and use tissue engineering concepts. One of the essential factors in pulp tissue engineering is a scaffold. In several reviews, direct bioactive material applied to the pulp without using scaffold will cause the temporary release, which was unstable. The scaffold can increase the success of pulp vital therapy treatment because the scaffold can facilitate stem cells to adhere, proliferate, differentiate and support regeneration. Hydrogel scaffold is considerable because it can mimicks extracellular matrix (ECM). It should have several essential characterizations, and one of them is biodegradable ability. New composite hydrogel scaffolds were developed as an organic and inorganic material hybrid. **Objective:** To compare the biodegradation value of Col-HA-EGCG hydrogel scaffold on days 3 and 7 after immersion. Materials and **Methods:** Samples were synthesized with the mixing of 1% hydroxyapatite solution and collagen solution until homogen, added 10 µmol/L EGCG into the solution. After that, 2% HPMC was used to stable the gelling process. Samples were freeze-dried for 24 hours and immersed in Phosphate Buffer Salin containing 1,6µg/ml of lysozyme enzyme. The degradation value percentages determined by measuring the difference weight of dry scaffold before and after immersion. **Results:** The data were analyzed by T-test, and it showed the Col-HA-EGCG hydrogel scaffold can be degraded, and there were no significant biodegradation values in 3 and 7 days. **Conclusions:** The Col-HA-EGCG is biodegradable in lysozyme enzyme. The biodegradation rate on Col-HA-EGCG scaffold on 3 and 7 days were not significant.

Keywords: biodegradable, hydrogel scaffold, Col-HA-EGCG, pulp dentin regeneration, regenerative endodontic

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INTRODUCTION

Preserving dental pulp vitality is a fundamental concept in conservative dentistry. General treatment of vital pulp therapy is pulp capping and pulpotomy. The common bioactive material used as pulp capping or pulpotomy agent is CaOH2. It can induce reparative dentin, but there was still a limitation, such as a tunnel defect causes pulp recontamination. It creates microleakage that allows microorganisms to penetrate to the pulp.(1) Therefore, the biological tissue engineering approach with scaffold seems to be considerable. The scaffold would provide a framework for the cell to adhere, proliferate, differentiate and grow to initiate the regenerative process. (2,3) Nowadays, hydrogel scaffold is also used with stem cells in dentin pulp complex regeneration. The sources of hydrogel scaffold can be classified into natural and synthetic. The advantages of natural sources are that they can deliver bioactive material, biocompatible, and biodegradable by a natural enzyme.(4) Natural sources can be obtained from bovine bone, fishbone, marine shells,and eggshells.(5–7) Formulating a hydrogel scaffold that can degrade in optimal time and in line with the tissue remodeling time was challenging.

Collagen (Col) is one of the considerable materials with excellent biocompatibility and biodegradability. It can form a hydrogel, which is appropriate for applying on rigid dentin.(8) Collagen can be used as tissue regeneration material because it can preserve the biological structure of the natural extracellular matrix.(9) Hydroxyapatite

(HA) and collagen can be combined with the sol-gel method to create a hydrogel scaffold because they are the main component of natural teeth, bone, and protein. Hydroxyapatite also has several advantages, such as osteoinductive, biocompatible, and non-toxic to the pulp tissue. (10–13) It can increase the Ca2+ concentration in the local area and also support proliferation, growth, and differentiation of mesenchymal stem cells.(14) To reduce the inflammation level of collagen and hydroxyapatite, Eppigallocathecin-3-Gallate (EGCG) can be used as crosslink material.(8,15) EGCG has a pleiotropic effect. It has an antioxidant effect and reduces inflammation to protect tissue from damage.(16)

Biodegradation of scaffold means the scaffold should be unraveled and eliminated from the body through a natural process. The scaffold should be resorbed in the control level and parallel with the growth of repairing tissue. Degradation rate can be affected by many factors, such as the structure and molecular weight of the polymer. A Scaffold should gradually be degraded, and it will be replaced by newly growing tissue from the attached cells. The result of the degradation process is the resorption through the surface of the scaffold. (17,18) Previous research has formulated a hydrogel scaffold from purified bovin collagen solution treated with different concentration of EGCG and it showed that 10µmol/L EGCG was biocompatible and has potential in increasing osteogenesis if there were other osteoinductive agent (8) So in this study, we used 1% nanohydroxyapatite as an ostoeconductive agent. (19) and formulated collagen solution, with the addition of EGCG. The final formulation was done by the addition of hydroxypropyl methyl cellulose (HPMC) 2% as a suspending agent that potential in providing sufficient setting time. This formulation is expected to take advantage of each material's properties, and it can be potentially used as pulp dentin regeneration material.

MATERIAL AND METHOD

Collagen used was bovine collagen type I (Gibco, Thermofisher Scientific), hydroxyapatite was taken from eggshell (Pro-db LC, BPertiwi Technology, Bogor Indonesia), and EGCG (Sigma Aldrich NoE4268 EGCG ≥80%). HMPC 2% (Benecel K100M, Ashland), Sodium Hydroxide 1 N, Phosphate Buffer Salin (Gibco, Thermofisher Scientific), deionized water, and sterile distilled water (Merck). Hydrogel scaffold formulation was made by dissolving hydroxyapatite with deionized water to create 1% hydroxyapatite solution in a magnetic stirrer for 1 hour at 350 rpm.(20) Meanwhile, collagen solution was prepared to 3 mg/ml concentration (1,8 ml) by adding 10x Phosphate Buffer Salin (PBS) (0.3 ml), 1 N Sodium hydroxide (0.045 ml), and steril distilled water (0.855 ml).(21) The hydroxyapatite and collagen solution was mixed together on cold condition. Then, 10µMol/L EGCG was added into the mixture solution, and it was stirred again until homogenous using cold temperature in magnetic stirrer.(22) After that, 2% HPMC was added until homogenous and formed colloidal at room temperature. (19,23) The formulation was frozen at -40 ° C for 2 hours and freeze-dried for 24 hours.(24) The measurement of hydrogel scaffold biodegradation was initiated by weighing the dry scaffold first. Then it was immersed in PBS with a 1,6µg/ml concentration of lysozyme enzyme. The concentration of the lysozyme enzyme was similar to the enzyme content in human serum. This PBS solution was replaced every day to ensure the continuity of enzyme activity. After the 3rd and 7th days, the samples are taken from immersion and washed with distilled water. Then the sample was freeze-dried again, and the dried scaffold was weighed again.(3,24) The biodegradation values were calculated by this formula (24–26):

$$\text{Degradation rate (\%)} = \frac{(W0-Wt)}{Wt} \times 100\%$$

Wo : Initial weight
Wt : Weight in t days

RESULTS

Data were analyzed using a T-test, and it showed no significant difference in biodegradation rate values on 3 and 7 days (P>0,05) The result analysis shown in table I.

Table I. Result Analysis of T-test to compare the differences of scaffold biodegradation value on days 3 and 7

Sample	Ratio	EGCG	HPMC 2%	Time (days)	Mean±Sd	P value
Col-HA-EGCG	1:1	10 µMol/L	0.05 mg	3	18.72 ±4.01	0.260
Col-HA-EGCG	1:1	10 µMol/L	0.05 mg	7	25.48% ±7.08	

The graphic result of collagen, hydroxyapatite and EGCG hydrogel scaffold with a 1:1 ratio and 10µMol/L EGCG is shown in figure 1. The biodegradation mean value of the scaffold on day 3 was 18.72±4.01 and 25.48%±7.08 on day 7. There was no difference in biodegradation values on days 3 and 7 statistically.

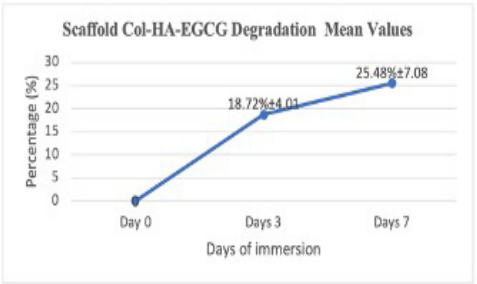

Fig.1 The graph of Col-HA-EGCG hydrogel scaffold degradation mean values

Fig. 1: The biodegradation mean value of the scaffold on day 3 was 18.72±4.01 and 25.48%±7.08 on day 7. Although even on day seven, the mean biodegradation value increased, there was no difference in biodegradation values on days 3 and 7 statistically. Data are given as mean±Sd (n=5)

DISCUSSION

The regeneration process of the pulp is initiated by the existance of stem cell from pulp origin that can differentiate into odontoblast like cell. Then, demineralized dentin can produce bioactive molecule that provide suitable microenvirontment for pulp regeneration. Acccording previous dentinogenesis study which used inducer biomaterial, the presence of mild and reversible inflammation usually exist in the pulp tissue on the 3rd days, and it decreased during days. The formation of mineralized tissue can be found on days 7.(28) Thus as an initial study, we used 3 and 7 days observation in scaffold biodegradation.

Nowadays, regenerative endodontic approaching in tissue engineering concept, and the scaffold is one of important factor. (29) Hydrogel scaffold can support the attachment of cell and provide the regeneration process by mimicking the pulp dentin extracellular matrix. It can perform the viscocity needed to be applicated in certain rigid dentin area to promote the regeneration process. (8,27)

The most essential in hydrogel scaffold formulations are suitable biomaterials that has a substantial effect on the success of tissue regeneration.(24) The novelty of this study is the component of scaffold material was based on bovine collagen, 1% hydroxyapatite sourced from eggshells, and EGCG formulated into a hydrogel. These bioactive materials expected be used as scaffolds to support pulp dentin regeneration treatment. The Triple helix structure of collagen is an essential property to biological interaction, and EGCG can be used to maintain collagen structure.(15) Hydroxyapatite has less mechanical property and it is difficult to be form as an injected material, so it composited with collagen which is natural polymer. The addition of collagen can promote endothelial cell and osteoblastic activity to form the new formation of pulp tissue.(30) To make a stable gel form, we need material such as hydroxypropyl methylcellulose (HPMC). HPMC can increase the viscocity of the composite, and collagen itself was unaltered in the addition of HPMC.(31) It also provide an enough time to gelation.(8)

In tissue engineering fields, biodegradable of a scaffold is crucial, because it shows viability of growth tissue. Too fast degradation process can affect the imperfect network formation due to loss of extraceluler matrix support. Otherwise, too slow degradation can activates immune response , and inhibits the tissue new formation. The biodegradation process should be linier with the remodelling process (4,27). In present study, it showed that scaffold Col-HA-EGCG can be degraded in PBS solution with lysozyme enzyme, therefore we also found that maybe it affected by the enzymatic activity and its stability in physiological tissue fluid. (32,33, 34) The value of biodegradation maybe also related to immersion time (day) and the concentration of HAp and collagen component.

In this study, The scaffold degradation rate performed weight reduction gradually on 0,3 and 7 days. The scaffold was reduce 18,72% and 25,48% from its original weight. It supports other study that the scaffold decreased weight gradually in certain time. The degradation mechanism focuses on enzymatic degradation using the lysozyme enzyme. In the human body, the enzyme lysozyme can be found in physiological fluids of the body, and also functions as an antibacterial and as a defense response against inflammation (35). In present scaffold contains collagens linked by hydrogen bonding and its interaction are not stable. It can be destroyed and dissociated by many factors, such as temperature, pH, enzymatic, and ionic bond. To increase the rate of biodegradation, covalent bonds are the recommended bonds. Crosslinking methods on collagen are divided into two categories: physical and chemical methods. This study used the chemical crosslinking method. Usually this method maybe has a toxic effect of residual crosslinking agent, so to overcome the disadvantage, the physical treatment maybe recommended, such as heat drying, UV and γ radiation. (36)

We found that the degradation value on days 3 and 7 was not significant statistically. It is possibly related with the hydroxyapatite composisition that needs more time to degraded, so more time observation should be developed. EGCG may also affect the property of the scaffold to not degrade easily. Previous study showed that the compressive strength, and surface roughness of scaffold is higher with the EGCG addition.(8)

The Col-HA-EGCG hydrogel scaffold was biodegradable, it can be seen by the reducing of scaffold weight after immersion in PBS solution. The ratio collagen and 1% hydroxyapatite determined because of the rational early formulation.(37), and according to previous study the higher hydroxyapatite will affect the setting time.(19)

According to the latest study, the higher concentration of hydroxyapatite would decrease the rate of biodegradation.(38), and on the contrary, the higher weight loss values was exist in the less HAp concentration.(33- 34) We found that 1 % HAp in this Col-HA-EGCG hydrogel composite was not related with the scaffold biodegradation value. Therefore, adding more or less hydroxyapatite concentration in collagen scaffold with EGCG formulation should be developed to prove the validity of the present theory.

The limitation of this study are the scaffold biodegradation was observed at 3 and 7 days. Biodegradation observation in 14 and 21 days is maybe required regarding to the regeneration and dentinogenesis times in actual condition and considers the time needed in tissue remodelling process. On this research , the

collagen and 1% hydroxyapatite with EGCG formulation performed insignificant result and we hadn't observed the other ratio, therefore further research can observe scaffold formulation in different ratios and days.

CONCLUSION

Novel hydrogel scaffold from collagen, hydroxyapatite, and EGCG has been successfully formulated and degraded in PBS solution contains lysozyme enzyme. The biodegradation rate on the collagen, 1% hydroxyapatite with EGCG on days 3 and 7 was not significant. Therefore, further observation of Col-HA-EGCG hydrogel scaffold with different HAp concentration and days should be considered.

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