21-Sep-2022

Dear Dr. Elline:

It is a pleasure to accept your manuscript entitled "Biodegradable Collagen, Hydroxyapatite, And Epigallocatechin-3-Gallate Hydrogel Scaffold As An Induction Material For Pulp Dentin Regeneration" in its current form for publication in the Malaysian Journal of Medicine & Health Sciences. The comments of the reviewer(s) who reviewed your manuscript are included at the foot of this letter.

Thank you for your fine contribution. On behalf of the Editors of the Malaysian Journal of Medicine & Health Sciences, we look forward to your continued contributions to the Journal.

Sincerely,
Dr. Normala Ibrahim
Editor-in-Chief, Malaysian Journal of Medicine & Health Sciences
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Associate Editor Comments to Author:

Associate Editor
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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.

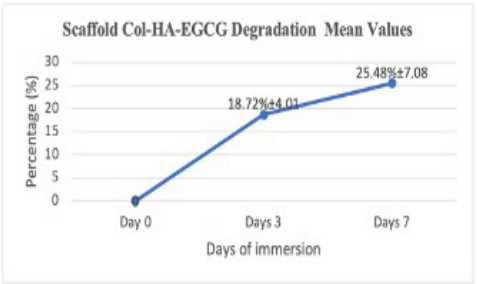

Fig.1 The graph of Col-HA-EGCG hydrogel scaffold degradation mean values

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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REFERENCES

1. Aprilia,Kunarti S, Budhi STI, Khoirunissa AP,Wulandari WOSA ,Sari RP, Rochyani LS. Stirring Time Effect of Beta-TCP Nanoencapsulation Synthesized from Anadara granosa Shells on Particle Size and Calcium Level. Mal J Med Health Sci. 2020;16(SUPP4):111–5. Available from : https://medic.upm.edu.my/upload/dokumen/2020070611290621_MJMHS_0156.pdf
2. Sharma A, Brand D, Fairbank J, Ye H, Lavy C, Czernuszka J. A self-organising biomimetic collagen/nano-hydroxyapatite-glycosaminoglycan scaffold for spinal fusion. J Mater Sci [Internet]. 2017 Nov [cited 2022 Jan 4];52(21):12574–92. DOI: 10.1007/s10853-017-1229-9
3. Widiyanti P, Gayatri DB, Rudyardjo DI. Synthesis and Characterization Scaffold Chitosan / Poly (ε-caprolactone) as Candidate for Skin Tissue Engineering in Burns. Mal J Med Health Sci. 2021;17(SUPP6): 24-29:6. Available from : https://medic.upm.edu.my/upload/dokumen/202109220035375)_2021_0264.pdf
4. Abbass MMS, El-Rashidy AA, Sadek KM, Moshy SE, Radwan IA, Rady D, et al. Hydrogels and Dentin–Pulp Complex Regeneration: From the Benchtop to Clinical Translation. Polymers [Internet]. 2020 Dec 9 [cited 2021 Jul 6];12(12):2935. DOI : 10.3390/polym12122935.
5. Afriani F. Perancah Berpori Hidroksiapatit Dan Β-Tricalcium Phosphate Dari Limbah Cangkang Telur Ayam Dengan Porogen Alginat. IPB. 2015;48. Available from: https://adoc.pub/sintesis-hidroksiapatit-berpori-dari-cangkang-telur-ayam-dan.html
6. Prahasti AE, Yuanita T. Utilization of Anchovy in Dentistry. Mal J Med Health Sci. 2021;17(SUPP6):87–90. Available from : https://medic.upm.edu.my/upload/dokumen/2021092200470214)_2021_0339.pdf
7. Anindyajati TP, Lastianny SP, Martien R, Murdiastuti K. The Effect of Cytotoxicity of Collagen-Chitosan Hydrogel on Platelets-Rich Plasma Various Formulation for Human Primary Fibroblast. Mal J Med Health Sci. 2020;16(SUPP15):45–50. Available from : https://medic.upm.edu.my/upload/dokumen/2020123012031308_MJMHS_0350.pdf
8. Kwon YS, Kim HJ, Hwang YC, Rosa V, Yu MK, Min KS. Effects of Epigallocatechin Gallate, an Antibacterial Cross-linking Agent, on Proliferation and Differentiation of Human Dental Pulp Cells Cultured in Collagen Scaffolds. Journal of Endodontics [Internet]. 2017 Feb [cited 2021 Sep 25];43(2):289–96. DOI: 10.1016/j.joen.2016.10.017
9. Dong C, Lv Y. Application of Collagen Scaffold in Tissue Engineering: Recent Advances and New Perspectives. Polymers [Internet]. 2016 Feb 4 [cited 2020 Oct 8];8(2):42. DOI: 10.3390/polym8020042
10. Sathiyavimal S, Vasantharaj S, LewisOscar F, Selvaraj R, Brindhadevi K, Pugazhendhi A. Natural organic and inorganic–hydroxyapatite biopolymer composite for biomedical applications. Progress in Organic Coatings [Internet]. 2020 Oct [cited 2021 Jan 17];147:105858. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0300944020308717
11. Okamoto M, Matsumoto S, Sugiyama A, Kanie K, Watanabe M, Huang H, et al. Performance of a Biodegradable Composite with Hydroxyapatite as a Scaffold in Pulp Tissue Repair. Polymers [Internet]. 2020 Apr 17 [cited 2020 Oct 13];12(4):937. DOI: 10.3390/polym12040937.
12. Elline E, Ismiyatin K. Nanohydroxiapatite Using Chicken Eggshell Waste and Its Characterization. Mal J Med Health Sci [Internet]. 2021;17(SUPP6):83–6. Available from: https://medic.upm.edu.my/upload/dokumen/2021092200460413)_2021_0338.pdf
13. Octarina O, Munadziroh E. Potential of Bovine Amniotic Membrane and Hydroxyapatite as Biocomposite Materials for Enhanced Bone Formation. Mal J Med Health Sci. 2021;17(SUPP6):124–6. Available from :https://medic.upm.edu.my/upload/dokumen/2021092200543520)_2021_0697.pdf
14. Filipović VV, Babić Radić MM, Vuković JS, Vukomanović M, Rubert M, Hofmann S, et al. Biodegradable Hydrogel Scaffolds Based on 2-Hydroxyethyl Methacrylate, Gelatin, Poly(β-amino esters), and Hydroxyapatite. Polymers [Internet]. 2021 Dec 22 [cited 2022 Mar 9];14(1):18. DOI: 10.3390/polym14010018
15. Chu C, Deng J, Man Y, Qua Y. Evaluation of nanohydroxyapaptite (nano-HA) coated

epigallocatechin-3-gallate (EGCG) cross-linked collagen membranes. Mater Sci Eng. 2017;78:258–64. DOI: 10.1016/j.msec.2017.04.069

16. Singh G, Kaur H, Harikumar SL. Pleiotropic Effects of Green Tea: An Overview. International Journal of Pharmaceutical and Phytopharmacological Research. 2015;223–6. Available from : https://eijppr.com/storage/models/article/3MC8F3YoB8rs8Z7eefIVVN2UkQ5C83MUD2shroNWXZrC1ZZ2nuLliiIQgf6d/pleiotropic-effects-of-green-tea-an-overview.pdf
17. Karakaya I, Ulusoy N, Department of Restorative Dentistry, Faculty of Dentistry, Near East University, Mersin 10, Turkey. Basics of dentin-pulp tissue engineering. AIMS Bioengineering [Internet]. 2018 [cited 2020 Oct 24];5(3):162–78. DOI: 10.3934/bioeng.2018.3.162
18. Herda E, Puspitasari D. Tinjauan Peran Dan Sifat Material Yang Digunakan Sebagai Scaffold Dalam Rekayasa Jaringan. JMKG. 2016;1(5):56–63. Available from : https://docplayer.info/94175635-Tinjauan-peran-dan-sifat-material-yang-digunakan-sebagai-scaffold-dalam-rekayasa-jaringan.html
19. Hikmawati D, Maulida HN, Putra AP, Budiatin AS, Syahrom A. Synthesis and Characterization of Nanohydroxyapatite-Gelatin Composite with Streptomycin as Antituberculosis Injectable Bone Substitute. International Journal of Biomaterials [Internet]. 2019 Jun 25 [cited 2021 Jul 11];2019:1–8. DOI : https://doi.org/10.1155/2019/7179243
20. Permatasari HA, Yusuf Y. Characteristics of Carbonated Hydroxyapatite Based on Abalone Mussel Shells (Halioitis asinina) Synthesized by Precipitation Method with Aging Time Variations. IOP Conf Ser: Mater Sci Eng [Internet]. 2019 Jun 1 [cited 2022 Feb 18];546(4):042031. doi:10.1088/1757-899X/546/4/042031
21. Gibco, Thermofisher. Collagen I, Bovine [Internet]. 2014. Available from: https://assets.fishersci.com/TFSAssets/LSG/manuals/A1064401_Bovine_collagen_I_PI.pdf
22. Zhao W, Liu Z, Liang X, Wang S, Ding J, Li Z, et al. Preparation and characterization of epigallocatechin-3-gallate loaded melanin nanocomposite (EGCG @MNPs) for improved thermal stability, antioxidant and antibacterial activity. LWT [Internet]. 2022 Jan [cited 2022 Jan 13];154:112599. DOI: https://doi.org/10.1016/j.lwt.2021.112599
23. Takallu S, Mirzaei E, Azadi A, Karimizade A, Tavakol S. Plate-shape carbonated hydroxyapatite/collagen nanocomposite hydrogel via in situ mineralization of hydroxyapatite concurrent with gelation of collagen at pH = 7.4 and 37°C. J Biomed Mater Res [Internet]. 2019 Aug [cited 2022 Jan 16];107(6):1920–9. DOI: 10.1002/jbm.b.34284
24. El Fadhlallah PM, Yuliati A, Soesilawati P, Pitaloka P. Biodegradation and Compressive Strength Test of Scaffold with Different Ratio as Bone Tissue Engineering Biomaterial. JIDMR [Internet]. 2018;11(2). Available from: http://www.jidmr.com
25. Zhou G, Liu S, Ma Y, Xu W, Meng W, Lin X, et al. Innovative biodegradable poly(L-lactide)/collagen/hydroxyapatite composite fibrous scaffolds promote osteoblastic proliferation and differentiation. IJN [Internet]. 2017 Oct [cited 2021 Dec 17];Volume 12:7577–88. DOI : 10.2147/IJN.S146679
26. Barbeck M, Serra T, Booms P, Stojanovic S, Najman S, Engel E, et al. Analysis of the in vitro degradation and the in vivo tissue response to bi-layered 3D-printed scaffolds combining PLA and biphasic PLA/bioglass components – Guidance of the inflammatory response as basis for osteochondral regeneration. Bioactive Materials [Internet]. 2017 Dec [cited 2020 Oct 14];2(4):208–23. DOI: 10.1016/j.bioactmat.2017.06.001
27. Zein N, Harmouch E, Lutz JC, Fernandez De Grado G, Kuchler-Bopp S, Clauss F, et al. Polymer-Based Instructive Scaffolds for Endodontic Regeneration. Materials [Internet]. 2019 Jul 24 [cited 2021 Jul 12];12(15):2347. Available from: https://www.mdpi.com/1996-1944/12/15/2347
28. Echeverria Molina MI, Malollari KG, Komvopoulos K. Design Challenges in Polymeric Scaffolds for Tissue Engineering. Front Bioeng Biotechnol [Internet]. 2021 Jun 14 [cited 2022 Jun 9];9:617141. DOI : https://doi.org/10.3389/fbioe.2021.617141
29. Baik SA, Mkenah AA, Khan A, Alkhalifah A, Makinah AA, Alquraini H, et al. Pulpotomy vs. pulpectomy techniques, indications and complications. International Journal of Community Medicine and Public Health. 2018;4. DOI: 10.18203/2394-6040.ijcmph20184261
30. Pankajakshan D, Voytik-Harbin SL, Nur JE, Bottino MC. Injectable Highly Tunable Oligomeric Collagen Matrices for Dental Tissue Regeneration. ACS Appl Bio Mater [Internet]. 2020 Feb 17 [cited 2022 Jun 8];3(2):859–68. DOI : https://doi.org/10.1021/acsabm.9b00944
31. Kesavan SK, Selvaraj D, Perumal S, Arunachalakasi A , Ganesan N, Chinnaiyan SK, Balaraman M. Fabrication of hybrid povidone-iodine impregnated collagen-hydroxypropyl methylcellulose composite scaffolds for wound-healing application. J Drug Deliv Sci Technol. 2022;70. DOI: 10.1016/j.jddst.2022.103247
32. Lee J, Yun H suk. Effect of hydroxyapatite-containing microspheres embedded into three-dimensional magnesium phosphate scaffolds on the controlled release of lysozyme and in vitro biodegradation. IJN [Internet]. 2014 Sep [cited 2022 May 5];4177. DOI: 10.2147/IJN.S68143
33. Yadav N, Srivastava P. In vitro studies on gelatin/hydroxyapatite composite modified with osteoblast for bone bioengineering. Heliyon [Internet]. 2019 May [cited 2021 Jan 25];5(5):e01633. DOI : https://doi.org/10.1016/j.heliyon.2019.e01633

34. Nitti P, Kunjalukkal Padmanabhan S, Cortazzi S, Stanca E, Siculella L, Licciulli A, et al. Enhancing Bioactivity of Hydroxyapatite Scaffolds Using Fibrous Type I Collagen. Front Bioeng Biotechnol [Internet]. 2021 Feb 4 [cited 2022 Aug 15];9:631177. DOI : 10.3389/fbioe.2021.631177
35. Pinheiro S, da Silva C, da Silva L, Cicotti M. Antimicrobial Capacity of a Hydroxyapatite–Lysozyme–Lactoferrin–Lactoperoxidase Combination Against Streptococcus mutans for the Treatment of Dentinal Caries. Indian J Dent Res [Internet]. 2020 [cited 2022 Aug 16];31(6):916. Available from: http://www.ijdr.in/text.asp?2020/31/6/916/311656
36. Dinescu S, Albu Kaya M, Chitoiu L, Ignat S, Kaya DA, Costache M. Collagen-Based Hydrogels and Their Applications for Tissue Engineering and Regenerative Medicine. In: Mondal MdIH, editor. Cellulose-Based Superabsorbent Hydrogels [Internet]. Cham: Springer International Publishing; 2018 [cited 2022 Aug 25]. p. 1–21. (Polymers and Polymeric Composites: A Reference Series). DOI: 10.1007/978-3-319-76573-0_54-1
37. Charlena, Ulum MF, Wati AK. Addition of hydroxypropyl methylcellulose to hydroxyapatite-chitosan composite as an injectable bone substitute. In Bogor, Indonesia; 2020 [cited 2022 Feb 15]. p. 030004. DOI : https://doi.org/10.1063/5.0004043
38. Tsutsui T. Dental Pulp Stem Cells: Advances to Applications. SCCAA [Internet]. 2020 Feb [cited 2021 Jan 8];Volume 13:33–42. DOI: 10.2147/SCCAA.S166759

21-Sep-2022

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Thank you for your fine contribution. On behalf of the Editors of the Malaysian Journal of Medicine & Health Sciences, we look forward to your continued contributions to the Journal.

Sincerely,
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Associate Editor Comments to Author:

Associate Editor
Comments to the Author:
(There are no comments.)

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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.

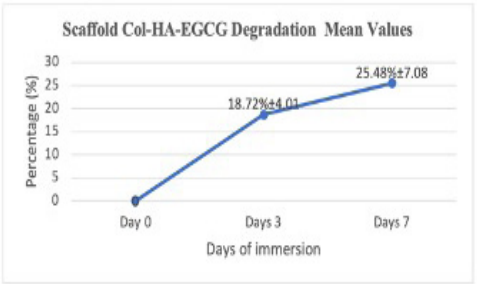

Fig.1 The graph of Col-HA-EGCG hydrogel scaffold degradation mean values

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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REFERENCES

1. Aprilia,Kunarti S, Budhi STI, Khoirunissa AP,Wulandari WOSA ,Sari RP, Rochyani LS. Stirring Time Effect of Beta-TCP Nanoencapsulation Synthesized from Anadara granosa Shells on Particle Size and Calcium Level. Mal J Med Health Sci. 2020;16(SUPP4):111–5. Available from : https://medic.upm.edu.my/upload/dokumen/2020070611290621_MJMHS_0156.pdf
2. Sharma A, Brand D, Fairbank J, Ye H, Lavy C, Czernuszka J. A self-organising biomimetic collagen/nano-hydroxyapatite-glycosaminoglycan scaffold for spinal fusion. J Mater Sci [Internet]. 2017 Nov [cited 2022 Jan 4];52(21):12574–92. DOI: 10.1007/s10853-017-1229-9
3. Widiyanti P, Gayatri DB, Rudyardjo DI. Synthesis and Characterization Scaffold Chitosan / Poly (ε-caprolactone) as Candidate for Skin Tissue Engineering in Burns. Mal J Med Health Sci. 2021;17(SUPP6): 24-29:6. Available from : https://medic.upm.edu.my/upload/dokumen/202109220035375)_2021_0264.pdf
4. Abbass MMS, El-Rashidy AA, Sadek KM, Moshy SE, Radwan IA, Rady D, et al. Hydrogels and Dentin–Pulp Complex Regeneration: From the Benchtop to Clinical Translation. Polymers [Internet]. 2020 Dec 9 [cited 2021 Jul 6];12(12):2935. DOI : 10.3390/polym12122935.
5. Afriani F. Perancah Berpori Hidroksiapatit Dan Β-Tricalcium Phosphate Dari Limbah Cangkang Telur Ayam Dengan Porogen Alginat. IPB. 2015;48. Available from: https://adoc.pub/sintesis-hidroksiapatit-berpori-dari-cangkang-telur-ayam-dan.html
6. Prahasti AE, Yuanita T. Utilization of Anchovy in Dentistry. Mal J Med Health Sci. 2021;17(SUPP6):87–90. Available from : https://medic.upm.edu.my/upload/dokumen/2021092200470214)_2021_0339.pdf
7. Anindyajati TP, Lastianny SP, Martien R, Murdiastuti K. The Effect of Cytotoxicity of Collagen-Chitosan Hydrogel on Platelets-Rich Plasma Various Formulation for Human Primary Fibroblast. Mal J Med Health Sci. 2020;16(SUPP15):45–50. Available from : https://medic.upm.edu.my/upload/dokumen/2020123012031308_MJMHS_0350.pdf
8. Kwon YS, Kim HJ, Hwang YC, Rosa V, Yu MK, Min KS. Effects of Epigallocatechin Gallate, an Antibacterial Cross-linking Agent, on Proliferation and Differentiation of Human Dental Pulp Cells Cultured in Collagen Scaffolds. Journal of Endodontics [Internet]. 2017 Feb [cited 2021 Sep 25];43(2):289–96. DOI: 10.1016/j.joen.2016.10.017
9. Dong C, Lv Y. Application of Collagen Scaffold in Tissue Engineering: Recent Advances and New Perspectives. Polymers [Internet]. 2016 Feb 4 [cited 2020 Oct 8];8(2):42. DOI: 10.3390/polym8020042
10. Sathiyavimal S, Vasantharaj S, LewisOscar F, Selvaraj R, Brindhadevi K, Pugazhendhi A. Natural organic and inorganic–hydroxyapatite biopolymer composite for biomedical applications. Progress in Organic Coatings [Internet]. 2020 Oct [cited 2021 Jan 17];147:105858. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0300944020308717
11. Okamoto M, Matsumoto S, Sugiyama A, Kanie K, Watanabe M, Huang H, et al. Performance of a Biodegradable Composite with Hydroxyapatite as a Scaffold in Pulp Tissue Repair. Polymers [Internet]. 2020 Apr 17 [cited 2020 Oct 13];12(4):937. DOI: 10.3390/polym12040937.
12. Elline E, Ismiyatin K. Nanohydroxiapatite Using Chicken Eggshell Waste and Its Characterization. Mal J Med Health Sci [Internet]. 2021;17(SUPP6):83–6. Available from: https://medic.upm.edu.my/upload/dokumen/2021092200460413)_2021_0338.pdf
13. Octarina O, Munadziroh E. Potential of Bovine Amniotic Membrane and Hydroxyapatite as Biocomposite Materials for Enhanced Bone Formation. Mal J Med Health Sci. 2021;17(SUPP6):124–6. Available from :https://medic.upm.edu.my/upload/dokumen/2021092200543520)_2021_0697.pdf
14. Filipović VV, Babić Radić MM, Vuković JS, Vukomanović M, Rubert M, Hofmann S, et al. Biodegradable Hydrogel Scaffolds Based on 2-Hydroxyethyl Methacrylate, Gelatin, Poly(β-amino esters), and Hydroxyapatite. Polymers [Internet]. 2021 Dec 22 [cited 2022 Mar 9];14(1):18. DOI: 10.3390/polym14010018
15. Chu C, Deng J, Man Y, Qua Y. Evaluation of nanohydroxyapaptite (nano-HA) coated

epigallocatechin-3-gallate (EGCG) cross-linked collagen membranes. Mater Sci Eng. 2017;78:258–64. DOI: 10.1016/j.msec.2017.04.069

16. Singh G, Kaur H, Harikumar SL. Pleiotropic Effects of Green Tea: An Overview. International Journal of Pharmaceutical and Phytopharmacological Research. 2015;223–6. Available from : https://eijppr.com/storage/models/article/3MC8F3YoB8rs8Z7eefIVVN2UkQ5C83MUD2shroNWXZrC1ZZ2nuLliiIQgf6d/pleiotropic-effects-of-green-tea-an-overview.pdf
17. Karakaya I, Ulusoy N, Department of Restorative Dentistry, Faculty of Dentistry, Near East University, Mersin 10, Turkey. Basics of dentin-pulp tissue engineering. AIMS Bioengineering [Internet]. 2018 [cited 2020 Oct 24];5(3):162–78. DOI: 10.3934/bioeng.2018.3.162
18. Herda E, Puspitasari D. Tinjauan Peran Dan Sifat Material Yang Digunakan Sebagai Scaffold Dalam Rekayasa Jaringan. JMKG. 2016;1(5):56–63. Available from : https://docplayer.info/94175635-Tinjauan-peran-dan-sifat-material-yang-digunakan-sebagai-scaffold-dalam-rekayasa-jaringan.html
19. Hikmawati D, Maulida HN, Putra AP, Budiatin AS, Syahrom A. Synthesis and Characterization of Nanohydroxyapatite-Gelatin Composite with Streptomycin as Antituberculosis Injectable Bone Substitute. International Journal of Biomaterials [Internet]. 2019 Jun 25 [cited 2021 Jul 11];2019:1–8. DOI : https://doi.org/10.1155/2019/7179243
20. Permatasari HA, Yusuf Y. Characteristics of Carbonated Hydroxyapatite Based on Abalone Mussel Shells (Halioitis asinina) Synthesized by Precipitation Method with Aging Time Variations. IOP Conf Ser: Mater Sci Eng [Internet]. 2019 Jun 1 [cited 2022 Feb 18];546(4):042031. doi:10.1088/1757-899X/546/4/042031
21. Gibco, Thermofisher. Collagen I, Bovine [Internet]. 2014. Available from: https://assets.fishersci.com/TFSAssets/LSG/manuals/A1064401_Bovine_collagen_I_PI.pdf
22. Zhao W, Liu Z, Liang X, Wang S, Ding J, Li Z, et al. Preparation and characterization of epigallocatechin-3-gallate loaded melanin nanocomposite (EGCG @MNPs) for improved thermal stability, antioxidant and antibacterial activity. LWT [Internet]. 2022 Jan [cited 2022 Jan 13];154:112599. DOI: https://doi.org/10.1016/j.lwt.2021.112599
23. Takallu S, Mirzaei E, Azadi A, Karimizade A, Tavakol S. Plate-shape carbonated hydroxyapatite/collagen nanocomposite hydrogel via in situ mineralization of hydroxyapatite concurrent with gelation of collagen at pH = 7.4 and 37°C. J Biomed Mater Res [Internet]. 2019 Aug [cited 2022 Jan 16];107(6):1920–9. DOI: 10.1002/jbm.b.34284
24. El Fadhlallah PM, Yuliati A, Soesilawati P, Pitaloka P. Biodegradation and Compressive Strength Test of Scaffold with Different Ratio as Bone Tissue Engineering Biomaterial. JIDMR [Internet]. 2018;11(2). Available from: http://www.jidmr.com
25. Zhou G, Liu S, Ma Y, Xu W, Meng W, Lin X, et al. Innovative biodegradable poly(L-lactide)/collagen/hydroxyapatite composite fibrous scaffolds promote osteoblastic proliferation and differentiation. IJN [Internet]. 2017 Oct [cited 2021 Dec 17];Volume 12:7577–88. DOI : 10.2147/IJN.S146679
26. Barbeck M, Serra T, Booms P, Stojanovic S, Najman S, Engel E, et al. Analysis of the in vitro degradation and the in vivo tissue response to bi-layered 3D-printed scaffolds combining PLA and biphasic PLA/bioglass components – Guidance of the inflammatory response as basis for osteochondral regeneration. Bioactive Materials [Internet]. 2017 Dec [cited 2020 Oct 14];2(4):208–23. DOI: 10.1016/j.bioactmat.2017.06.001
27. Zein N, Harmouch E, Lutz JC, Fernandez De Grado G, Kuchler-Bopp S, Clauss F, et al. Polymer-Based Instructive Scaffolds for Endodontic Regeneration. Materials [Internet]. 2019 Jul 24 [cited 2021 Jul 12];12(15):2347. Available from: https://www.mdpi.com/1996-1944/12/15/2347
28. Echeverria Molina MI, Malollari KG, Komvopoulos K. Design Challenges in Polymeric Scaffolds for Tissue Engineering. Front Bioeng Biotechnol [Internet]. 2021 Jun 14 [cited 2022 Jun 9];9:617141. DOI : https://doi.org/10.3389/fbioe.2021.617141
29. Baik SA, Mkenah AA, Khan A, Alkhalifah A, Makinah AA, Alquraini H, et al. Pulpotomy vs. pulpectomy techniques, indications and complications. International Journal of Community Medicine and Public Health. 2018;4. DOI: 10.18203/2394-6040.ijcmph20184261
30. Pankajakshan D, Voytik-Harbin SL, Nur JE, Bottino MC. Injectable Highly Tunable Oligomeric Collagen Matrices for Dental Tissue Regeneration. ACS Appl Bio Mater [Internet]. 2020 Feb 17 [cited 2022 Jun 8];3(2):859–68. DOI : https://doi.org/10.1021/acsabm.9b00944
31. Kesavan SK, Selvaraj D, Perumal S, Arunachalakasi A , Ganesan N, Chinnaiyan SK, Balaraman M. Fabrication of hybrid povidone-iodine impregnated collagen-hydroxypropyl methylcellulose composite scaffolds for wound-healing application. J Drug Deliv Sci Technol. 2022;70. DOI: 10.1016/j.jddst.2022.103247
32. Lee J, Yun H suk. Effect of hydroxyapatite-containing microspheres embedded into three-dimensional magnesium phosphate scaffolds on the controlled release of lysozyme and in vitro biodegradation. IJN [Internet]. 2014 Sep [cited 2022 May 5];4177. DOI: 10.2147/IJN.S68143
33. Yadav N, Srivastava P. In vitro studies on gelatin/hydroxyapatite composite modified with osteoblast for bone bioengineering. Heliyon [Internet]. 2019 May [cited 2021 Jan 25];5(5):e01633. DOI : https://doi.org/10.1016/j.heliyon.2019.e01633

34. Nitti P, Kunjalukkal Padmanabhan S, Cortazzi S, Stanca E, Siculella L, Licciulli A, et al. Enhancing Bioactivity of Hydroxyapatite Scaffolds Using Fibrous Type I Collagen. Front Bioeng Biotechnol [Internet]. 2021 Feb 4 [cited 2022 Aug 15];9:631177. DOI : 10.3389/fbioe.2021.631177
35. Pinheiro S, da Silva C, da Silva L, Cicotti M. Antimicrobial Capacity of a Hydroxyapatite–Lysozyme–Lactoferrin–Lactoperoxidase Combination Against Streptococcus mutans for the Treatment of Dentinal Caries. Indian J Dent Res [Internet]. 2020 [cited 2022 Aug 16];31(6):916. Available from: http://www.ijdr.in/text.asp?2020/31/6/916/311656
36. Dinescu S, Albu Kaya M, Chitoiu L, Ignat S, Kaya DA, Costache M. Collagen-Based Hydrogels and Their Applications for Tissue Engineering and Regenerative Medicine. In: Mondal MdIH, editor. Cellulose-Based Superabsorbent Hydrogels [Internet]. Cham: Springer International Publishing; 2018 [cited 2022 Aug 25]. p. 1–21. (Polymers and Polymeric Composites: A Reference Series). DOI: 10.1007/978-3-319-76573-0_54-1
37. Charlena, Ulum MF, Wati AK. Addition of hydroxypropyl methylcellulose to hydroxyapatite-chitosan composite as an injectable bone substitute. In Bogor, Indonesia; 2020 [cited 2022 Feb 15]. p. 030004. DOI : https://doi.org/10.1063/5.0004043
38. Tsutsui T. Dental Pulp Stem Cells: Advances to Applications. SCCAA [Internet]. 2020 Feb [cited 2021 Jan 8];Volume 13:33–42. DOI: 10.2147/SCCAA.S166759