DENTINO
JURNAL KEDOKTERAN GIGI
Vol VIII. No 2. September 2023

THE EFFECTIVENESS OF CHITOSAN NATURAL SOURCES ON PULP TISSUE REGENERATION

Aisya Nadhifa Ahmad[1)], Muhammad Yanuar Ichrom Nahzi[2)], Juli Harnida Purwaningayu[3)]
[1)] Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin
[2)] Department of Conservative Dentistry, Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin
[3)]Department of Biomedical Dentistry, Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin

ABSTRACT
Background: *Dental pulp plays a role in forming dentin, providing nutrition, and sensing harmful stimuli to the tooth. Pulp damage can occur due to physical, chemical, and biological factors. A regenerative therapy was developed so that the pulp can regenerate its tissue and the teeth can survive in the oral cavity with vital conditions. Tissue engineering is a principle developed in the treatment of pulp tissue regeneration. The main elements of tissue engineering are stem cells, scaffolds, and growth factors. Scaffolds are elements that support cell organization, migration, proliferation, differentiation, and vascularization. One example of a biomaterial that can be used as a scaffold is chitosan. Chitosan has been proved to be suitable as a biomedical material because it has several beneficial properties including increasing cell proliferation, attachment, and differentiation.* ***Purpose:*** *This study aims to determine the most effective natural ingredient chitosan for pulp tissue regeneration.* ***Methods:*** *This study used the literature review method with a narrative review procedure. The literature was searched using Google Scholar, Science Direct, ProQuest, and PubMed.* ***Results:*** *The results of the study from 6 articles showed that chitosan from shrimp, crab, and mushroom had the potential to increase pulp tissue regeneration in terms of 3 indicators (cell proliferation, attachment, and differentiation).* ***Conclusion:*** *The most effective chitosan from natural sources for pulp regeneration is shrimp chitosan and mushroom chitosan.*

Keywords: *Chitosan, Natural Sources, Pulp Tissue Regeneration*
Corespondence: Aisya Nadhifa Ahmad; Faculty of Dentistry, University Lambung Mangkurat, Veteran Street No. 128B, Banjarmasin 70249, South Kalimantan, Indonesia. Email: anaaisya4669@gmail.com

INTRODUCTION

The dental pulp is a soft tissue derived from mesenchyme located in the center of the tooth whose constituent components consist of cells, fibers, interstitial fluid, odontoblasts, and fibroblasts. Odontoblasts and other cells found in the pulp can differentiate into hard tissue, making the pulp play a role in dentin formation. The pulp and dentin have a relationship called the dentin-pulp complex which has a regenerative potential that leads to the formation of tertiary dentin in response to abnormal stimuli, such as excessive tooth wear, cavity preparation, restorative materials, and caries.[1,2] In addition, the pulp also plays a role in providing nutrition and sensing noxious stimuli, such as pain from temperature, extreme pressure, or trauma to the dentin and pulp. These stimuli can have an impact on pulp damage.[3]

Pulp damage can occur due to pulp exposure due to physical factors, chemical factors, and biological factors.[4] These factors will cause an inflammatory response. During the inflammatory reaction, there is the release of lysosomal enzymes that cause hydrolysis of collagen and the release of kinins. Further changes lead to increased vascular permeability. The fluid that comes out accumulates in the pulp chamber, as a result the pressure in the pulp chamber increases due to the limited space in the pulp causing pain.[1]

Root canal treatment is one of the endodontic treatments where endodontic treatment is considered an effective treatment for irreversible pulp.[5] However, endodontic treatment will cause the teeth to become non-vital and more brittle than when the teeth were still vital. This can happen because after endodontic treatment there is no longer vascularization and innervation to the tooth, and the pulp will lose its ability to form reparative dentin. In addition, endodontic treatment procedures will also increase the possibility of root fractures, so it is necessary to develop a new therapy whose aim is to regenerate pulp tissue so that

the teeth can survive in the oral cavity in a vital condition.[4]

A regenerative therapy was developed so that the pulp can regenerate its tissue and the teeth can survive in the oral cavity with vital conditions. Regenerative therapy for dentin or pulp is an ideal therapy to improve the function and morphology of teeth that are impaired by pulp injury or inflammation.[4] Tissue engineering is a principle developed in regenerative therapy and is defined as a procedure designed based on engineering and scientific principles to develop biologics that replace, maintain, or improve tissue function. The main elements of tissue engineering are stem cells, scaffolds, and growth factors. Tissue engineering technology in the field of endodontics is known as regenerative endodontics.[6,7]

The regenerative endodontics technique is divided into several strategies, one of those is cell homing. Cell homing is a tissue regeneration technique that uses the principles of cell recruitment and differentiation. Recruitment refers to the migration of stem cells to the injured site, whereas differentiation refers to the transformation of stem cells into mature cells.[8] Four stages occur in cell homing. The first stage is chemotaxis, where stem cells migrate from the apical region. This happens because it is induced by a scaffold containing growth factors. After the chemotaxis stage, the cells begin to proliferate with the increasing number of scaffolds containing growth factors, this is the second stage. The third stage cells have attached to the root canal surface and the fourth stage cells differentiate into pulp-like cells.[9]

One of the main elements of regenerative endodontics is the scaffold. Scaffold is an element that provides support for cell organization, migration, proliferation, differentiation, and vascularization. The requirements for the scaffold to be used are non-toxic, porous to allow placement of cells, biocompatible with host tissues, biodegradable, and must be degraded gradually so that it is replaced by regenerative tissue. One example of a biomaterial that can be used as a scaffold is chitosan.[3,10]

Chitosan is a polysaccharide derived from chitin and modified by the deacetylation process.[11] Chitosan is non-toxic and has a high molecular weight. This polysaccharide has proven to be suitable as an ideal material for biomedical applications due to its antimicrobial properties, fungistatic, biocompatibility, biodegradability, non-carcinogenicity, as well as promotion of cell adhesion, proliferation, and differentiation. Based on these properties, several studies have used chitosan as a biomaterial, including in the development of scaffolds for tissue engineering, and more specifically for pulp regeneration.[3,12] Chitosan is found in natural and synthetic forms. However, natural chitosan has advantages compared to synthetic chitosan, it has good biocompatibility and biodegradability properties, as well as wide availability.[13] Chitosan natural ingredients can be obtained from the cuticle or exoskeleton of insects, arthropods, molluscs, and crustaceans.[14]

Several existing studies explain the different effectiveness of one type of chitosan natural sources for pulp tissue regeneration, so researchers are interested in conducting a literature review because no one has ever summarized the comparison of the effectiveness of chitosan from various natural sources on pulp tissue regeneration. Researchers used three stages of cell homing as an indicator of pulp regeneration, there are cell proliferation, attachment, and differentiation.

RESEARCH METHOD

The method used in this study is literature review. Literature review is a survey of scientific articles, books, and other sources relevant to a particular problem, field of research, or theory, and provides a description, summary, and critical evaluation of these works. The purpose of a literature review is to update the previous literature on a topic and form the basis for other purposes such as justification for future research in the field.[15]

The keywords used in this study were, “chitosan”, “pulp regeneration”, “regenerative endodontics treatment”, and “tissue engineering” as well as those related to these keywords. Literature searches were carried out using Google Scholar, Proquest, and Science Direct. The criteria for the reviewed sources are reputable English sources Q1, Q2, and Q3 through the SJR Scimago ranking (SCImago Journal & Country Rank), which are publications between 2011-2021 and can be accessed in full text.

RESULT

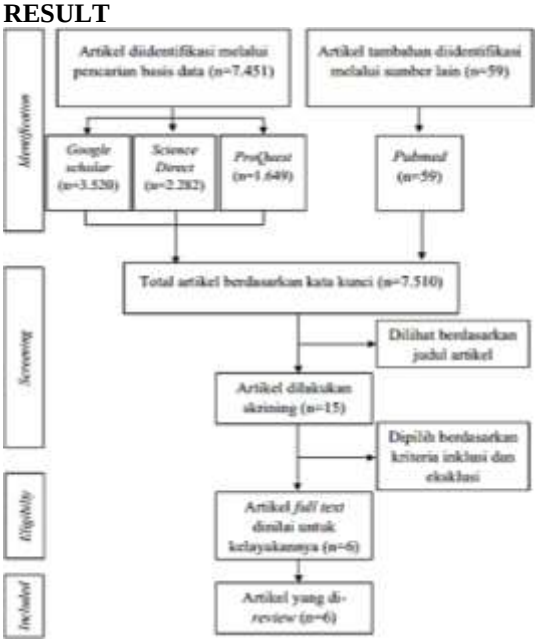

Figure 1. Prism Diagram of Literature Review

The results of the literature search found 6 articles that were worthy of review including 3 articles using shrimp chitosan, 1 article using crab chitosan, and 2 articles using fungal chitosan. The 6 articles stated that chitosan affects cell proliferation, attachment, and differentiation.

Table 1. Pulp Tissue Regeneration Indicator

Article	Chitosan	Indicator		
		Proliferation	Attachment	Differentiation
Ducret et al	Shrimp	*	-	*
Flores et al	Shrimp	*	*	*
Amir et al	Shrimp	*	-	*
Sana et al	Crab	*	*	*
Abbas et al	Fungal	-	-	*
EzEldeen et al	Fungal	-	*	-

Based on table 1, total of 6 articles were reviewed has 4 articles discussed cell proliferation, 3 articles discussed cell attachment, and 5 articles discussed differentiation. The results of the review obtained 3 natural sources of chitosan that can be used as a scaffold material, there are shrimp, crab, and fungal.

Table 2. Comparison of the Proliferation of Chitosan Natural Sources

Article	Chitosan	Result
Ducret et al	Shrimp	(chitosan-fibrin)The highest cell proliferation occurred on the 4th day
Flores et al	Shrimp	(ChCDG) The highest cell proliferation occurred on the 8th day
Amir et al	Shrimp	(Ch 5µg/mL) The highest cell proliferation occurred on the 14th day
Sana et al	Crab	(Ch+Fn) The highest cell proliferation occurred on the 5th day

Based on table 2, it was found that 3 articles used shrimp chitosan and 1 article used crab chitosan. The proliferation value with the fastest time is in the chitosan-fibrin group of shrimp chitosan on day 4.

Table 3. Comparison of the Attachment of Chitosan Natural Sources

Article	Chitosan	Result
Flores et al	Shrimp	The ChGDG group showed that there was a spread of cells with filopodia on the surface of the scaffold on the 4th day and covered the entire surface of the scaffold on the 8th day.
Sana et al	Crab	The chitosan-RGD group showed more adherent cells compared to the chitosan group after one day. In the chitosan-fibronectin group, it was found that there was attachment and spread on the surface of the scaffold on the 1st and 9th days.
EzEldeen et al	Fungal	The fCS-GEL-GPTMS group showed the best attachment compared to other groups, judging from the metabolic activity of the 7th-day cells.

Based on table 3, it was found that the journals used different natural ingredients, there are shrimp, crab, and fungal. The fCS-GEL-GPTMS group had the fastest cell attachment on the 7th day.

Table 4. Comparison of the Differentiation of Chitosan Natural Sources

Article	Chitosan	Result
Ducret et al	Shrimp	(chitosan-fibrin) The highest cell differentiation occurred on the 7th day
Flores et al	Shrimp	(ChGDG) The highest cell differentiation occurred on the 21st day
Amir et al	Shrimp	(Ch 10 µg/mL) The highest cell differentiation occurred on the 7th day
Sana et al	Crab	(Cn+Fn) The highest cell differentiation occurred on the 14th day
Abbas et al	Fungal	All groups did not increase the formation of dentinal hard tissue on the canal walls

Based on table 4, it was found that 3 articles used chitosan shrimp, 1 article used chitosan crab, and 1 article used chitosan shrimp. The value of differentiation with the fastest time was found in chitosan shrimp in the Ch 10 g/mL group on the 7th day and in the ChGDG group on the 7th day.

DISCUSSION

The Effectiveness of Chitosan Based on Cell Proliferation

The total articles reviewed were 6 articles, which 3 articles tested shrimp chitosan on cell proliferation.

The results of the review showed that cell proliferation can be seen from the expression of the MKI67 gene and the absorbance value. MKI67 is a protein that appears in the cell cycle and can be used as a marker to determine cell proliferation.[16] The first article was written by Ducret et al (2019) which discussed the effect of chitosan-fibrin scaffold on cell proliferation as seen from the expression of MKI67.[17] The results in the article stated that the highest expression of the MKI67 gene in the fibrin-chitosan group was on day 4. Fibrin added to the scaffold has a role in pulp regeneration. Ruwangsawasdi et al (2014) also mentioned that fibrin helps cell proliferation and differentiation for the formation of new pulp tissue and found that the newly formed pulp tissue is very similar to the normal pulp tissue.[18]

The MTT assay is the most common assay to measure cell viability and proliferation.[19] The MTT assay functions to measure cell viability by looking at the absorbance value, the higher the absorbance value, the higher the cell viability. Viability values that exceed the control group indicate the occurrence of cell proliferation.[20] The second article was written by Flores et al (2021) which discussed the effect of chitosan scaffold added with gelatin, genipin, and microparticulate dentin on cell proliferation using the MTT assay.[21] The chitosan-gelatin-microparticulate dentin-genipin (ChGDG) group on day 8 had the highest absorbance value compared to the chitosan (Ch) group and the chitosan-gelatin-genipin (ChGG) group. The absorbance value of the ChGDG group was also higher than the control group, which means that there has occurred cell proliferation in the ChGDG group. The high cell proliferation in the ChGDG group could occur because the addition of gelatin, microparticulate dentin, and genipin was able to increase cell adhesion. The higher the cell attachment, the higher the cell proliferation that occurs.[18-20] In addition, proteins in dentin can increase cell proliferation on the scaffold.[22]

The third article was written by Amir et al (2014) which discussed the effect of the chitosan group at concentrations of 5 and 10 g/mL on cell proliferation.[23] The MTT assay is also used in research to determine the value of cell proliferation. The results showed that the highest absorbance value was found in the 5 g/mL group of chitosan on day 14. If the 5 g/mL chitosan group was compared with the Ch group in the article by Flores et al (2021), the Ch group had lower cell proliferation. This situation could occur due to the use of different stem cells, hDPSC, and hCAP, resulting in different results. The concentration of chitosan used can also affect the results of cell proliferation. Amir et al (2014) used 5 and 10 g/m, while Flores et al (2021) did not mention the concentration of chitosan used.[21,23] Based on the results of the review, the most effective group of shrimp shell chitosan based on cell proliferation was the chitosan-fibrin group in DP-MSC because it only needed 4 days of culture to achieve high proliferation.

There is 1 article that tested crab chitosan on the value of cell proliferation. This article was written by Sana et al (2014) which discussed the effect of chitosan scaffold added with fibronectin on cell proliferation.[24] The test used to determine the value of cell proliferation is the resazurin test which is measured by the absorbance value based on the reduction of resazurin to resofurin by the metabolic activity of living cells.[25] The higher the absorbance value, the higher the cell viability. Sana et al (2017) stated that the highest absorbance value was in the Ch+Fn group on day 5 of hDPSC. This could be due to the addition of fibronectin in the Ch+Fn group to promote better adhesion and assist in cell proliferation. Fibronectin itself is a protein that plays a role in proliferation, attachment, and differentiation.[26]

Based on the results of the review, the chitosan-fibrin group from shrimp chitosan only needed 4 days of culture to achieve the highest proliferation, while crab chitosan took 5 days, so it was concluded that shrimp chitosan was considered more effective for pulp tissue regeneration in terms of cell proliferation. Further research is needed to determine the effect of using different stem cells and different chitosan concentrations on cell proliferation.

The Effectiveness of Chitosan Based on Cell Attachment

There is 1 article that tested shrimp chitosan on cell attachment. The article of Flores et al (2021) discussed the effect of chitosan scaffold added with gelatin, genipin, and microparticulate dentin on cell adhesion which can be seen using SEM.[21] SEM is a type of electron microscope that can produce high-resolution images of a sample surface, so the images produced by SEM have qualitative characteristics in two dimensions because they use electrons instead of light waves and useful for determining the surface structure of the sample.[27] The surface description of the scaffold in the ChGDG group showed that there was a spread of cells with filopodia on the surface of the scaffold on the 1st day and covered the entire surface on the 9th day. The addition of gelatin and microparticulate dentin was able to increase cell adhesion and surface roughness of the scaffold, thereby increasing cell attachment to the scaffold.[18-20]

There is 1 article that tested crab chitosan on cell attachment. This article was written by Sana et al (2014) which discussed the effect of chitosan scaffold added with fibronectin on cell adhesion.[24] The test used to determine cell attachment is SEM. The chitosan-fibronectin group was found to have attachment and spread on the surface of the scaffold on day 1 to day 9. The addition of fibronectin to the Ch+Fn group promotes better adhesion.[26]

There is 1 article that tested fungal chitosan on cell attachment, the article was written by EzEldeen (2021). The test used in this article to determine cell

attachment is the Alamar Blue test measured by the metabolic activity of cells.[28] The cell's metabolic activity indirectly conveys information about attachment or proliferation.[29] The best cell adhesion was in the fCS-GEL-GPTMS group on day 7 which could occur due to the addition of RGD to gelatin which could increase cell adhesion.[30]

Based on the results of the review, the ChGDG group (shrimp chitosan) and the fCS-GEL-GPTMS group (fungal chitosan) produced better cell attachment than the Ch+Fn group (crab chitosan). The ChGDG group took 8 days and the fCS-GEL-GPTMS group took 7 days to complete cell attachment.

The Effectiveness of Chitosan Based on Differentiation

There are 3 articles that test shrimp chitosan on cell differentiation. The results of the review showed that cell differentiation could be seen from the expression of odontogenic marker (COL1A1) and mineralized nodule formation. The article by Ducret et al (2019) and the article by Amir et al (2014) used the COL1A1 gene to determine the value of cell differentiation.[17,23] The COL1A1 gene is a gene that orders the manufacture of type I collagen constituent proteins which are important components of the ECM.[31,32] Ducret et al stated that the highest expression of COL1A1 was in the fibrin-chitosan group on day 7. Amir et al said that the expression of COL1A1 was found in the chitosan group of 10 g/mL on the 7th day. The results of these two studies prove that chitosan is able to initiate the formation of type I collagen.

The article by Flores et al (2021) used the formation of mineralized nodules to determine the value of cell differentiation which could be analyzed using the Alizarin red test. The alizarin red test is a hard bone staining test that serves to detect the calcification process in the stained bone area.[33] The article by Flores et al (2021) stated that the Ch and ChGG groups interfere with the formation of the ECM.[21] However, the ChGDG group was able to increase COL1A1 expression more than the control group. It can be concluded that the addition of dentin acts as an osteoconductive which increases the mineralized tissue. In addition, the addition of genipin was also able to promote the formation of mineralized tissue.[34]

There is 1 article that tested crab chitosan on cell differentiation. This article was written by Sana et al (2014) who discussed the effect of chitosan scaffold added with fibronectin on cell differentiation by looking at the expression of COL1A1.[24] COL1A1 gene expression in the chitosan-fibronectin group decreased during the culture period. Research by Lia et al (2014) also stated that there was a decrease in the expression of the COL1A1 gene towards the end of the culture period.[35]

The article of Abbas et al (2020) showed that the chitosan-demineralized bone matrix (REG-CD) group and the chitosan-dexamethasone group did not increase the formation of mineralized tissue along the canal and no histological tissue formation was found.[36]

Based on the results of the review, shrimp chitosan has the ability to differentiate compared to crab chitosan and fungal chitosan. shrimp Chitosan had a high differentiation value after 7 days of culture, while crab chitosan required 21 days and fungal chitosan did not increase differentiation in pulp tissue. It can be concluded that shrimp chitosan has better effectiveness than crab and fungal chitosan. Based on the research that has been done, the most effective chitosan from natural sources is shrimp and fungal chitosan. All natural chitosan sources (shrimp, crab, and fungal) have potential as scaffold materials in regenerative endodontic treatment. Further research is needed on the effectiveness of natural ingredients chitosan with different concentrations and different stem cells.

REFERENCES

1. Garg N & Garg A. Textbook of Endodontics. Jaypee Brothers. 2014. p5-7.
2. Yendriwati, Lindawati Y, Nababan RS, Oral DB, Gigi FK, Utara US. Distribusi Dentin Tersier pada Puncak Pulpa Gigi. dentika Dent J. 2016;19(1):1–3.
3. Coimbra P, Alves P, Valente TAM, Santos R, Correia IJ, Ferreira P. Sodium Hyaluronate/Chitosan Polyelectrolyte Complex Scaffolds for Dental Pulp Regeneration: Synthesis and Characterization. Int J Biol Macromol. 2011;49(4):573–579.
4. Sugiaman VK. Peranan Sel Punca dalam Meregenerasi Jaringan Pulpa Gigi. Sonde. 2019;4(1):43–51.
5. Fouad AF. The microbial challenge to pulp regeneration. Adv Dent Res. 2011;23(3):285–289.
6. Jamal M. Dental Stem Cells and Their Potential Role in Regenerative Medicine. J Med Sci. 2011;4(2):53–61.
7. Sangappa SK, Javanaiahar N, Kumar AP, Shruti S. Regenerative endodontic: current progress. IOSR J Dent Med Sci. 2014;13(4):88–95.
8. He L, Zhong J, Gong Q, Cheng B, Kim SG, Ling J, Mao JJ. Regenerative Endodontics by Cell Homing. Dent Clin North Am. 2017;61(1):143–159.
9. Münchow EA, Bottino MC. Current and Future Views on Biomaterial Use in Regenerative Endodontics. Clin Approaches Endod Regen. 2019;77–98.
10. Goldberg M. The Dental Pulp. Springer. 2014. p251-254.
11. Marieta A, Musfiroh I. Review Artikel : Berbagai Aktivitas Farmakologi dari Senyawa Kitosan. Farmaka. 2019;17(2):105–110.
12. Kania D, Putri T, Oktiani BW, Adhani R. Antioxidant Activity Potency Of Chitosan From Haruan (Channa Striata) Scales. Dentino. 2020;5(2):139-144.
13. Fakhri E, Eslami H, Maroufi P, Pakdel F, Taghizadeh S, Ganbarov K, Yousefi M, Tanomand A, Yousefi B, Mahmoudi S, Kafil HS. Chitosan biomaterials application in dentistry. Int J Biol Macromol. 2020;162:956–974.
14. Sukpaita T, Chirachanchai S, Pimkhaokham A, Ampornaramveth RS. Chitosan-Based Scaffold for Mineralized Tissues Regeneration. Marine Drugs. 2021;19(551):1–14.
15. Ramdhani A, Ramdhani MA, Amin AS. Writing a

Literature Review Research Paper: A step-by-step approach. Int J Basic Appl Sci. 2014;3(1):47–56.
16. Xiong DD, Zeng CM, Jiang L, Luo DZ, Chen G. Ki-67/MKI67 as a predictive biomarker for clinical outcome in gastric cancer patients: An Updated Meta-analysis and Systematic Review involving 53 Studies and 7078 Patients. J Cancer. 2019;10(22):5339–5354.
17. Ducret M, Montembault A, Josse J, Pasdeloup M, Celle A, Benchrih R, Gerin FM, Licht BA, David, L, Farges JC. Design and characterization of a chitosan-enriched fibrin hydrogel for human dental pulp regeneration. Dent Mater. 2019;35(4):523–533.
18. Ruangsawasdi N, Zehnder M, Weber FE. Fibrin gel improves tissue ingrowth and cell differentiation in human immature premolars implanted in rats. J Endod. 2014;40(2):246–250.
19. Vega-Avila E, Pugsley MK. An overview of colorimetric assay methods used to assess survival or proliferation of mammalian cells. Proc West Pharmacol Soc. 2011;54(March 2014):10–14.
20. Vitria RDN, Yuanita T, Pribadi N. Uji Viabilitas Flavonoid Ekstrak Kulit Buah Manggis (Garcinia mangostana L.) terhadap Sel Fibroblas BHK-21. Jurnal Conserv Dent. 2015;5(2):26–31.
21. Caballero-Flores H, Nabeshima CK, Sarra G, Moreira MS, Arana-Chavez VE, Marques MM, Mchado MEDL. Development and characterization of a new chitosan-based scaffold associated with gelatin, microparticulate dentin, and genipin for endodontic regeneration. Dent Mater. 2021;37(7):e414–425.
22. Galler KM, Brandl FP, Kirchhof S, Widbiller M, Eidt A, Buchalla W, Göpferich A, Schmalz G. Suitability of Different Natural and Synthetic Biomaterials for Dental Pulp Tissue Engineering. Tissue Eng - Part A. 2018;24(3–4):234–244.
23. Amir LR, Suniarti DF, Utami S, Abbas B. Chitosan as a potential osteogenic factor compared with dexamethasone in cultured macaque dental pulp stromal cells. Cell Tissue Res. 2014;358(2):407–415.
24. Asghari Sana F, Çapkın Yurtsever M, Kaynak Bayrak G, Tunçay EÖ, Kiremitçi AS, Gümüşderelioğlu M. Spreading, proliferation and differentiation of human dental pulp stem cells on chitosan scaffolds immobilized with RGD or fibronectin. Cytotechnology. 2017;69(4):617–630.
25. Bafadal M, Mutiara WO, Malaka MH, Fristiohady A, Yodha WM, Sadarun B, Sahidin I. Aktivitas Sitotoksik Ekstrak Etanol Petrosia sp. Secara In Vitro terhadap Sel Kanker Serviks HeLa. Jurnal Farmasi Sains dan Praktis. 2021;7(3):282–288.
26. Liu D, Che Abdullah CA, Sear RP, Keddie JL. Cell adhesion on nanopatterned fibronectin substrates. Soft Matter. 2010;6(21):5408–5416.
27. Cahyana A, Marzuki A. Analisa SEM (Scanning Electron Microscope) pada Kaca TZN yang dikristalkan Sebagian. Pros Math Sci Forum. 2014;232–236.
28. Ezeldeen M, Loos J, Mousavi Nejad Z, Cristaldi M, Murgia D, Braem A, Jacobs R. 3D-Pri Nti Ng-Assi Sted Fabri Cati on of Chi Tosan Scaffolds From Different Sources and Cross-Linkers for Dental Tissue Engineering. Eur Cells Mater. 2021;41:485–501.
29. Zhou X, Holsbeeks I, Impens S, Sonnaert M, Bloemen V, Luyten F, Schrooten J. Noninvasive real-time monitoring by alamarBlue® during in vitro culture of three-dimensional tissue-engineered bone constructs. Tissue Eng - Part C Methods. 2013;19(9):720–727.
30. Kumar P, Dehiya BS, Sindhu A. Comparative study of chitosan and chitosan–gelatin scaffold for tissue engineering. Int Nano Lett. 2017;7(4):285–290.
31. Majchrzycki M, Bartkowiak-Wieczorek J, Wolski H, Drews K, Bogacz A, Czerny B, Ulan EZ, Mrozikiewicz AS. Polymorphisms of collagen 1A1 (COL1A1) gene and their relation to bone mineral density in postmenopausal women. Ginekol Pol. 2015;86(12):907–914.
32. Kartika, Suadiatmika DGM. Gambaran Radiologi untuk Deteksi Prenatal Osteogenesis Imperfecta. Cdk. 2018;45(6):446–448.
33. Bakri A, Pujiliningsih E, Hambali M, Kanedi M. Pengaruh Lama Perendaman Alizarin Red Terhadap Kadar Warna Rangka Fetus Mencit (Mus musculus L) untuk Bahan Praktikum dan Penelitian di Laboratorium. Int Multiling J Sci Technol. 2020;5(11):1925–1929.
34. Kwon YS, Lim ES, Kim HM, Hwang YC, Lee KW, Min KS. Genipin, a cross-linking agent, promotes odontogenic differentiation of human dental pulp cells. J Endod. 2015;41(4):501–507.
35. Abbas KF, Tawfik H, Hashem AAR, Ahmed HMA, Abu-Seida AM, Refai HM. Histopathological evaluation of different regenerative protocols using Chitosan-based formulations for management of immature non-vital teeth with apical periodontitis: In vivo study. Aust Endod J. 2020;46(3):405–414.
36. Palma PJ, Ramos JC, Martins JB, Diogenes A, Figueiredo MH, Ferreira P, Viegas C, Santos JM. Histologic Evaluation of Regenerative Endodontic Procedures with the Use of Chitosan Scaffolds in Immature Dog Teeth with Apical Periodontitis. J Endod. 2017;43(8):1279–1287.