The Effect of Chitosan on the Formation of Odontoblast-Like Cells in Reversible Pulpitis (in Vivo Study on Sprague Dawley Rats)

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Abstract. Reversible pulpitis is inflammation of the pulp that can change back to the normal pulp. The medicament is critical to the success of direct pulp capping treatment, as evidenced by the formation of odontoblast-like cells. Calcium hydroxide has begun to be abandoned because of its deficiency. This study aims to determine the effect of chitosan-coated red snapper fish scales as a medicament for direct pulp capping on the formation of odontoblast-like cells. This study examined the effect of chitosan on the formation of odontoblast-like cells in vivo. The samples used were from 4 groups: healthy rats, reversible pulpitis rats, reversible pulpitis rats treated with calcium hydroxide, and reversible pulpitis rats treated with chitosan. Observation of odontoblast-like cells was carried out using an Optilab Olympus CX23 microscope with a magnification of 1000 times and was calculated using image raster software. The results showed the formation of odontoblast-like cells with an average of in group 1 0.166, group 2 0.066, group 3 0.600, and group 4 0.633. Then the Kruskal Wallis test was carried out, and the results obtained were $p = 0.006$ ($p < 0.05$), meaning a significant difference. The results of the Mann-Whitney test showed that there were significant and insignificant differences. Based on this study, it can be concluded that chitosan red snapper fish scales used as a direct pulp capping material influence the formation of odontoblast-like cells in reversible pulpitis.

Keywords: Reversible pulpitis · Chitosan red snapper scales · Direct pulp cap · Odontoblast-like cell

1 Introduction

Pulpitis is an inflamed pulp [1] Pulpitis is classified into reversible pulpitis and irreversible pulpitis. Reversible pulpitis is a mild to moderate inflammatory condition of the pulp, whereas irreversible pulpitis is a continuation of reversible pulpitis. The pulp

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with reversible pulpitis can return to a normal pulp if the irritant stimulus is removed [2]. Pulps with irreversible pulpitis cannot return to a normal pulp because the pulp has a necrosis [3] Reversible pulpitis that is not treated promptly has the risk of causing pulpal death and tooth loss [4].

Characteristics of the healing process of exposed pulp tissue are the remodeling of damaged soft tissue, the formation of odontoblast-like cells, and the formation of a reparative dentine [5]. Direct pulp capping treatment is carried out by placing medicaments in cavities with exposed pulp to maintain pulp vitality [6]. Medicament suppresses the inflammatory process by inducing the formation of odontoblast-like cells [7] Odontoblast-like cells will form reparative dentin, which will cover the exposed pulp so that it can protect the pulp from more severe damage [8] The ideal requirements for medicaments include stimulating reparative dentine formation, maintaining the pulp as vital, being antibacterial, adhering to dentine and restorative materials, and being able to act as a barrier to prevent bacteria from entering [9].

One of Indonesia's abundant marine products is red snapper. The protein content in red snapper fish scales can be extracted into chitin and then converted into chitosan through deacetylation. Chitosan is non-toxic, biocompatible, and biodegradable, making it suitable for the biomedical field [10] Research using chitosan as a hemostatic agent in pulpotomy treatment has proven that chitosan can increase the formation of reparative dentine and hard tissue [11]. 1The higher degree of deacetylation of chitosan can increase the formation of odontoblast-like cells, which play a role in the healing process of pulp tissue [12]. This study aims to determine the effect of chitosan from red snapper fish scales on the formation of odontoblast-like cells in pulp tissue with reversible pulpitis.

2 . Research Methods

The sample used was 24 Sprague Dawley rats, which were divided into four groups consisting of group 1, healthy rats, group 2, reversible pulpitis rats, group 3, reversible pulpitis rats applied calcium hydroxide, and group 4, reversible pulpitis rats applied chitosan. Chitosan is made from red snapper fish scales obtained from the market. The scales of the red snapper are washed thoroughly and then dried. The dried fish scales were mashed with a blender and then sieved using an 80-mesh sieve to produce a fine powder.

2.1 Chitosan Manufacture

The process of isolating red snapper scales starts at the deproteinized stage: putting 125 g of red snapper scale powder into a beaker glass. Red snapper scale powder was added with 3.5% (w/v) NaOH at a ratio of 1:10. The mixture was heated at 70 °C accompanied by stirring for 1 h using a magnetic stirrer then filtered with filter paper and washed with distilled water to obtain a neutral pH. The solid obtained is dried in an oven at 60 °C for 1 h or until it has a constant weight.

The second stage is demineralization, which is carried out by adding solids with 1N HCL at a ratio of 1:10 in a beaker glass. The mixture was allowed to stand at room temperature and then heated to 75 °C, accompanied by stirring for 1 h using a magnetic

stirrer, and then filtered with filter paper and washed with distilled water to obtain a neutral pH. The solid, thus obtained is dried in an oven at 60 °C for 1 h or until it has a constant weight. The resulting product is chitin.

The third stage is deacetylation, which is carried out by adding chitin powder to 3.5% NaOH with a ratio of 1:10 in a beaker glass. The mixture was heated to 75 °C and stirred for 1 h using a magnetic stirrer, then filtered with filter paper and washed with distilled water to obtain a neutral pH. The solid thus obtained is dried in an oven at 60 °C for 1 h or until it has a constant weight. The product produced at this stage is red snapper fish scale chitosan. The degree of chitosan deacetylation was calculated using the Fourier Transform Infrared (FTIR) spectrophotometry method. The analysis showed that the degree of deacetylation of the chitosan scales of red snapper was 91.126%.

2.2 Experimental Animal Treatment

Sprague Dawley rats were anesthetized intramuscularly using 0.2 cc of ketamine. The surface of the maxillary first molar was opened using a round diamond bur with a cavity diameter of 2 mm and a depth of 1 mm. The pulp roof was perforated using a sonde and then checked using a k-file. The cavity on the teeth of the rats was treated with a plastic instrument, according to the group. Group 2 was applied with cavity, group 3 was applied with calcium hydroxide, and group 4 was applied with chitosan.

After the 28th day, the euthanasia process was performed by placing the rats in an airtight container filled with cotton containing chloroform. Dead rats were subjected to jaw cutting using surgical scissors to remove their teeth. Maxillary first molars were taken and put in a sealed container containing 10% formalin buffer solution. Furthermore, the decalcification process was carried out by immersing the tissue in HCL for seven days. Tissue processing was carried out in several stages: dehydration, clearing, embedding, tissue cutting, and tissue staining using hematoxylin-eosin.

Odontoblast-like cells were observed in 5 randomly selected fields of view using an Optilab Olympus CX23 microscope with 1000x magnification and counted using image raster software. Each visual field is scored based on the parameters described in Table 1 [13].

The data obtained were then tested statistically using SPSS 22 software. The data were subjected to the Shapiro-Wilk test to determine their normal distribution and the homogeneity test, namely Levene's test. If the test results show that the data is normally distributed and homogeneous, it meets the requirements for a one-way ANOVA parametric test.

Table 1. Scores and Parameters of odontoblast-like cells

Styles	Parameters
1	The number of odontoblast-like cells in each visual field 1–20
2	The number of odontoblast-like cells in each visual field 21–40
3	The number of odontoblast-like cells in each visual field 41–80

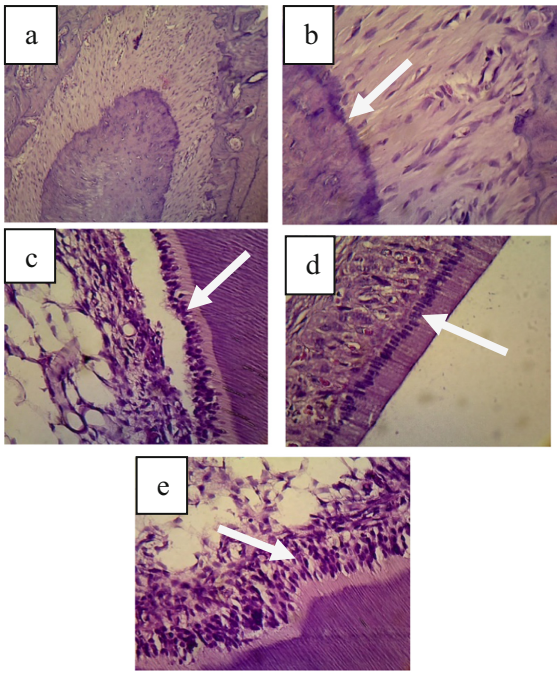

Fig. 1. a) Odontoblast-like cell score 0, b) Odontoblast-like cell score 1, c) Odontoblast-like cell score 2, d) Odontoblast-like cell score 3, e) Odontoblast-like cell score 4.

3 Results

Based on the observations of odontoblast-like cells, the average number of odontoblast-like cells in each group is shown in Table 2.

Table 2. The average result of odontoblast-like cells The average results showed that the most odontoblast-like cell formation occurred in group 4, while the lowest odontoblast-like cell formation occurred in group 2.

The results of the Shapiro-Wilk test for groups 1, 2, and 4 have a p-value < 0.05, so the data is not normally distributed. Levene's test results obtained a value of $p = 0.240$, where the value of $p > 0.05$ indicates homogeneous data. The one-way ANOVA test does not apply to data that is not normally distributed. An alternative test that can be performed if data normality is not met is the Kruskal-Wallis test. The results of the Kruskal Wallis test obtained $p = 0.006$, so the $p < 0.05$ indicated a significant difference between treatment groups. The Mann-Whitney test was performed to determine the significant relationship between groups. The Mann-Whitney test results can be seen in Table 3.

Table 3 Mann-Whitney Test Results*Sig < 0.05, indicating a significant difference in the number of odontoblast-like cell formations.

Based on the results of the Mann-Whitney test, it was found that there was a significant difference in the formation of odontoblast-like cells ($p < 0.05$) in groups 1 to 3, groups 1 to 4, groups 2 to 3, and groups 2 to group 4.

4 Discussion

Trauma to the rat teeth causes the pulpal roof to open. The exposed pulp becomes an entry point for microorganisms into the pulp, causing inflammation [14]. Inflammation that occurs will make the original odontoblast cells carry out a defensive reaction, namely the ability to carry out recovery. In mild pulp tissue damage, the original odontoblast cells that are not damaged will form reactionary dentin. However, with more severe damage, the original odontoblast cells will die and be replaced by odontoblast-like cells [15].

The medicaments used in treating pulp caps will help stimulate an increase in the number of progenitor cells. Progenitor cells function to stimulate an increase in transforming growth factor-beta 1 (TGF-β1), which is the initial stage in the formation of odontoblast-like cells produced by undifferentiated cells of the dental papilla mesenchyme [16]. Chitosan and calcium hydroxide can both stimulate the formation of odontoblast-like cells. However, the formation of odontoblast-like cells carried out by calcium hydroxide is inconsistent [17]. The high pH of calcium hydroxide can help release proteins and growth factors (TGF-β1) [18] On the other hand, the strong base of calcium hydroxide can cause mitochondrial dysfunction in tissues. These conditions will increase superoxide radicals that diffuse through the cytosol and then enter the mitochondria, resulting in decreased function. If the mitochondria die, the cell will lack oxygen and eventually die [19].

Pulp healing depends on the ability of the medicament to form an excellent seal to prevent the entry of microorganisms into the pulp [20]. Chitosan has a better sealing ability than calcium hydroxide because it is a polyelectrolyte that can interact with the surface and negative biomolecules. The amine group in chitosan can bind to the hydroxyl particles in dentin [21, 22]. The ability of chitosan to form a good seal can prevent microorganism contamination from entering the pulp, preventing inflammation and promoting pulp repair.

The rats treated with chitosan had the highest average number of odontoblast-like cell formations among the other groups. This is because the chitosan in red snapper fish scales has a high degree of deacetylation. A high degree of deacetylation will increase the biocompatibility properties of chitosan in the wound healing process, especially in the formation of odontoblast-like cells [23, 24]. The active compound N-acetyl D-glucosamine in chitosan will cross-link with glycosaminoglycans and glycoproteins, which play a role in activating TGF-β1. Chitosan will enhance the role of TGF-β1, which stimulates the formation of odontoblast-like cells, by protecting TGF-β1 so that it is not easily degraded by enzymes and heat [25]. Chitosan will also accelerate wound healing through its ability to bind heparin polyanions to form polyelectrolyte complexes [26] Heparin slows the diffusion of TGF-1 in the tissues, increasing its half-life [27].

5 Conclusion

Through the research that has been done, it can be concluded that chitosan from red snapper scales used as a pulp cap medicament can increase the amount of odontoblast-like cell formation in reversible pulpitis.

References

1. J. R. Ningsih, *Ilmu Dasar kedokteran Gigi*. Muhammadiyah University Press, 2018
2. S. Dewiyani and E. J. Palupi, "Distribusi Frekuensi Pulpitis Reversibel dan Pulpitis Ireversibel di RSGM FKG Moestopo Pada Tahun 2014-2016 (Berdasarkan Jenis Kelamin, Usia dan Lokasi Gigi)," *J. Ilm. dan Teknol. Kedokt. Gigi*, vol. 15, no. 2, pp. 41–46, 2019, https://doi.org/10.32509/jitekgi.v15i2.892.
3. D. Ricucci, S. Loghin, and J. F. Siqueira, "Correlation between clinical and histologic pulp diagnoses," *J. Endod.*, vol. 40, no. 12, pp. 1932–1939, 2014, https://doi.org/10.1016/j.joen.2014.08.010.
4. J. Salis and S. Kaswindiarti, "Perawatan Pulpotomi Pada Gigi Desidui Posterior Maksila: Laporan Kasus," *Pros. Dent. Semin. Univ. Muhammadiyah Surakarta*, pp. 93–105, 2020
5. I. Dwiandhono, R. Effendy, and S. Kunarti, "The thickness of odontoblast-like cell layer after induced by propolis extract and calcium hydroxide," *Dent. J. (Majalah Kedokt. Gigi)*, vol. 49, no. 1, pp. 17–21, 2016, https://doi.org/10.20473/j.djmkg.v49.i1.p17-21
6. J. R. Ningsih and N. Rachmadani, "Comparison Between Mineral Trioxide Aggregate (Mta) and Biodentine As Direct Pulp Capping Material : a Literature Review,"*Pros. Temu Ilm. Nas. IKORGI V*, pp. 458–465, 2021
7. E. Octiara, "Dentin Reparatif Dan Growth Factor Yang Berperan Dalam Dentinogenesis Reparatif (Reparative Dentin and the Role of Growth Factor in Reparative Dentinogenesis)," *dentika Dent. J.*, vol. 18, no. 3, pp. 294–299, 2015
8. M. Goldberg, "Central Indirect and Direct Pulp Capping: Reactionary vs. Reparative Dentins," *JSM Dent*, vol. 8, no. 1, pp. 1–6, 2020.
9. A. Qureshi, E. Soujanya, N. Kumar, P. Kumar, and S. Hivarao, "Recent advances in pulp capping materials: An overview," *J. Clin. Diagnostic Res.*, vol. 8, no. 1, pp. 316–321, 2014, https://doi.org/10.7860/JCDR/2014/7719.3980
10. B. T. Hartomo and F. G. Firdaus, "Pemanfaatan Biomaterial Kitosan Dalam Bidang Bedah Mulut," *B-Dent J. Kedokt. Gigi Univ. Baiturrahmah*, vol. 6, no. 1, pp. 62–70, 2019.
11. B. Erpaçal, Ö. Adigüzel, S. Cangül, and M. Acartürk, "A General Overview of Chitosan and its Use in Dentistry," *Int. Biol. Biomed. J.*, vol. 5, no. 1, pp. 1–11, 2019.
12. W. Prananingrum, "The increasing of odontoblast-like cell number on direct pulp capping of Rattus norvegicus using chitosan," *Dent. J. Maj. Kedokt. Gigi*, vol. 43, no. 4, pp. 168–171, 2010.
13. Y. He *et al.*, "Pulpal Tissue Inflammatory Reactions after Experimental Pulpal Exposure in Mice," *J. Endod.*, vol. 43, no. 1, pp. 90–95, 2017, https://doi.org/10.1016/j.joen.2016.09.003.
14. A. Sumidarti and S. N. Moersidi, "Reversible pulpitis accompanied with sinus tract on buccal side of the right maxillary first premolar teeth: case report," *J. Dentomaxillofacial Sci.*, vol. 4, no. 2, p. 120, 2019. https://doi.org/10.15562/jdmfs.v4i2.969
15. E. O. Orhan, M. Maden, and B. Senguüven, "Odontoblast-like cell numbers and reparative dentine thickness after direct pulp capping with platelet-rich plasma and enamel matrix derivative: A histomorphometric evaluation," *Int. Endod. J.*, vol. 45, no. 4, pp. 317–325, 2012. https://doi.org/10.1111/j.1365-2591.2011.01977.x
16. G. R. Ravi and R. V. Subramanyam, "Possible mechanisms of lack of dentin bridge formation in response to calcium hydroxide in primary teeth," *Dent. Hypotheses*, vol. 6, no. 1, pp. 6–9, 2015. https://doi.org/10.4103/2155-8213.150863.
17. T. J. Hilton, "Keys to Clinical Success with Pulp Capping: A Review of the Literature," *Oper. Dent.*, vol. 34, no. 5, pp. 615–625, 2009. https://doi.org/10.2341/09-132-0
18. X. V. Tran *et al.*, "Effect of a calcium-silicate-based restorative cement on pulp repair," *J. Dent. Res.*, vol. 91, no. 12, pp. 1166–1171, 2012. https://doi.org/10.1177/0022034512460833

19. I. Nirwana, E. Munadziroh, R. Yogiartono, C. Thiyagu, C. Ying, and A. DInaryanti, "Cytotoxicity and proliferation evaluation on fibroblast after combining calcium hydroxide and ellagic acid," *J. Adv. Pharm. Technol. Res.*, vol. 12, no. 1, pp. 27–31, 2021. https://doi.org/10.4103/japtr.JAPTR_154_20
20. C. Cox, G. Bogen, H. Kopel, and J. Ruby, "Repair of Pulpal Injury by Dental Materials In: Hagreaves KM," *Quintessence Publ.*, vol. 5, no. 6, pp. 1–6, 2002
21. E. Sagita and S. Surini, "Pembuatan Matriks dari Kompleks Polielektrolit Kitosan Pektin untuk Sediaan Tablet Mengapung," *Pharm. Sci. Res.*, vol. 7, no. 3, 2010. https://doi.org/10.7454/psr.v7i3.3462
22. H. Sutrisman, T. Abidin, and H. Agusnar, "Pengaruh chitosan belangkas (Tachypleus gigas) nanopartikel terhadap celah antara berbagai jenis semen ionomer kaca dengan dentin," *Dent. J. (Majalah Kedokt. Gigi)*, vol. 47, no. 3, pp. 121–125, 2014. https://doi.org/10.20473/j.djmkg.v47.i3.p121-125
23. M. Khoroushi, M. R. Foroughi, S. Karbasi, B. Hashemibeni, and A. A. Khademi, "Effect of Polyhydroxybutyrate/Chitosan/Bioglass nanofiber scaffold on proliferation and differentiation of stem cells from human exfoliated deciduous teeth into odontoblast-like cells," *Mater. Sci. Eng. C*, vol. 89, pp. 128–139, 2018. https://doi.org/10.1016/j.msec.2018.03.028
24. S. B. Nimbeni, B. S. Nimbeni, and D. D. Divakar, "Role of chitosan in remineralization of enamel and dentin: A systematic review," *Int. J. Clin. Pediatr. Dent.*, vol. 14, no. 4, pp. 562–568, 2021, https://doi.org/10.5005/jp-journals-10005-1971.
25. T. Niwa *et al.*, "The dynamics of TGF-β in dental pulp, odontoblasts and dentin," *Sci. Rep.*, vol. 8, no. 1, pp. 1–14, 2018. https://doi.org/10.1038/s41598-018-22823-7
26. F. Al Baani, R. A. Lusiana, and M. C. Djunaidi, "Pengaruh Agen Pencangkok Heparin terhadap Kemampuan Transpor Kreatinin dan Urea Membran Turunan Kitosan," *J. Kim. Sains dan Apl.*, vol. 20, no. 2, pp. 92–94, 2017. https://doi.org/10.14710/jksa.20.2.92-94
27. C. C. Rider and B. Mulloy, "Heparin, heparan sulphate and the TGF- Cytokine superfamily," *Molecules*, vol. 22, no. 5, pp. 1–11, 2017. https://doi.org/10.3390/molecules22050713