

## Effectiveness of three pulp capping materials on cellular response of pulp tissue

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### ABSTRACT

**Background:** Pulp capping is a treatment that utilizes medications to preserve pulp vitality.  $\text{Ca}(\text{OH})_2$ , mineral trioxide aggregate (MTA), and biodentine are presently the most common pulp capping medications. As pulp capping materials,  $\text{Ca}(\text{OH})_2$  and MTA are known to have several weaknesses. Biodentine has been created in an effort to mitigate the drawbacks of  $\text{Ca}(\text{OH})_2$  and MTA. Thus, the purpose of this study is to compare the efficacy of three pulp capping medications in reducing inflammation and accelerating pulp regeneration.

**Method:** Total 48 male Wistar rats, that were divided into 4 groups, namely 1<sup>st</sup> control group, 2<sup>nd</sup> MTA group, 3<sup>rd</sup> biodentine group, and 4<sup>th</sup>  $\text{Ca}(\text{OH})_2$  group. Maxillary 1<sup>st</sup> molar teeth of rats were prepared using round bur no. 080 perpendicular to the tooth axis to a depth of 1mm, until a reddish color appeared on the pulp. Furthermore, pulp capping medicament was applied to the deepest points, and temporary fillings were made with  $\text{Zn}_2(\text{PO}_4)_3$ . Decapitation was carried out on days 4 and 7 to count the number of neutrophil cells, macrophages, odontoblast-like cells, and fibroblasts. Data were analyzed using One-way ANOVA ( $p < 0.05$ ).

**Results:** The results demonstrated that there were significant differences between the three groups that were treated with pulp capping medications. The group treated with biodentine had the lowest number of neutrophil and macrophage cells and the highest number of odontoblast-like cells and fibroblasts, compared to the groups treated with  $\text{Ca}(\text{OH})_2$  and MTA.

**Conclusion:** Biodentine demonstrated efficacy as a pulp capping material, as indicated by a lower number of neutrophils and macrophages as inflammatory markers and a higher number of odontoblast-like cells and fibroblasts compared to the  $\text{Ca}(\text{OH})_2$  and MTA group.

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## INTRODUCTION

At the center of each tooth is pulp, a non-mineralized tooth tissue composed of cells, matrix, vascularization, and nerves. There are five primary functions of pulp, including inductive, formative, protective, nutritive, and reparative.<sup>1,2</sup> When the pulp becomes inflamed due to bacterial, physical, or chemical factors, it can lose its function. By treating the pulp capping, the vitality of the inflamed pulp can be maintained.<sup>3</sup>

Direct pulp capping is a treatment for exposed pulp with the intent to protect the pulp to ensure it remains vital and eliminates tissue irritation.<sup>4</sup> In this treatment, a regeneration process will begin with the activity of inflammatory cells by neutrophils and macrophages, the activity of odontoblast-like cells produced by mesenchymal cells, and the activity of fibroblast cells that generate collagen fibers, followed by the formation of reparative dentine.<sup>5</sup>

Calcium Hydroxide ( $\text{Ca(OH)}_2$ ) is one of the most frequently used medicines for fiber capping materials.<sup>6,7</sup> Since the 1920s, calcium hydroxide has been used in dentistry and is regarded as the "gold standard" for pulp capping treatment. This medication has antibacterial and alkaline properties. According to a number of studies, however, this material has a number of disadvantages and side effects, such as causing necrosis of the pulp's superficial layers and bacterial infection due to tunnel defects.<sup>8,7</sup> Tunnel defects are formed because these medicaments cannot adapt to dentine and have high solubility, causing fissures in the dentine, facilitating the penetration of bacteria and toxins which results in treatment failure and causes pulpal irritation.<sup>9</sup> Mineral trioxide aggregate (MTA) can also be used as a pulp closure material due to its ability to form dentinal bridges and oral cavity compatibility.<sup>7</sup> The thickness of the newly formed dentinal bridge was greater following pulp

encapsulation with MTA than with  $\text{Ca(OH)}_2$ .<sup>6</sup> However, MTA has a number of disadvantages, including a long setting time (more than 3 hours), unstable powder after packaging is unsealed (must be used within 4 weeks), and a high price tag.<sup>10</sup> Biodentine is a novel drug that can be used as pulp capping material. Pulp capping with biodentine medications has demonstrated favorable results in pulp healing and regeneration and has a positive effect compared to other current medications.<sup>11</sup> Thus, the purpose of this investigation is to compare the efficacy of three pulp capping medications (biodentine, MTA, and  $\text{Ca(OH)}_2$ ) based on the responses of neutrophils, macrophages, odontoblast-like cells, and fibroblasts.

## RESEARCH METHOD

The subjects of the study were 48 male Wistar rats divided into four groups: group I (control), group II (MTA), group III (Biodentine), and group IV ( $\text{Ca(OH)}_2$ ). with twelve rats in each cohort. The rats were anesthetized with ketamine HCL 10% 0.1ml/100g body weight intramuscularly in the thighs, and then the maxillary 1st molars of the right and left rats were prepared with round bur no. 080 perpendicular to the axis of the tooth 1mm deep until a reddish color was observed on the pulp.<sup>12</sup> Then, immediately following preparation, the medication was administered to the bleeding site. The hole was sealed with  $\text{Zn}_3(\text{PO}_4)_2$  cement. On day 4, 24 mice were decapitated to count neutrophil cells and macrophages, and on day 7, 24 mice were decapitated to count odontoblast-like cells and fibroblasts. Hematoxylin-eosin (HE) staining was performed and observed with a 1000X magnification microscope. One-way ANOVA was used to analyze the research data ( $p < 0.05$ ). No.142/B.1-KEPK/SA-FKG/2019 was issued by the Health Research Ethics Commission of the Faculty

of Dentistry at the Islamic University of Sultan Agung Semarang.

**RESULTS**

The biodentine group has the lowest average number of neutrophils and macrophages when compared to other groups (Table 1). In contrast to the other groups, the number of odontoblast-like cells and fibroblasts increased in the biodentine group.

According to the One Way ANOVA test (Table 2), there are significant differences between

treatment groups in the number of neutrophils, macrophages, odontoblast-like cells, and fibroblasts. The results of the Post Hoc Test (Table 3) revealed that there were significant differences in the counts of neutrophils, macrophages, odontoblast-like cells, and fibroblasts among all groups, with the exception of MTA and biodentine. Biodentine is as effective as MTA at reducing the number of neutrophils and macrophages and promoting the formation of odontoblast-like cells and fibroblasts.

**Table 1.** Average Number of Neutrophils, Macrophages, odontoblast-like Cells, and Fibroblasts

Groups	X ± SD			
	Neutrophils	Macrophages	Odontoblast Like Cells	Fibroblasts
Control (-)	6.83 ± 1.94	4.40 ± 1.14	3.33 ± 2.16	4.00 ± 1.10
Ca(OH) <sub>2</sub>	6.50 ± 2.58	4.00 ± 1.22	5.83 ± 1.72	5.50 ± 1.51
MTA	3.66 ± 1.50	2.40 ± 0.55	9.33 ± 2.42	7.17 ± 0.75
Biodentine	2.50 ± 0.54	1.80 ± 0.84	10. ± 0.89	8.17 ± 1.17

**Table 2.** One-Way ANOVA Test on Comparison of The Number of Neutrophils, Macrophages, Odontoblast-Like Cells, and Fibroblasts

Groups	P
Control (-)	0.000 Significant difference
Ca(OH) <sub>2</sub>	
MTA	
Biodentine	

**Table 3.** Post Hoc LSD Test on Comparison of The Number of Neutrophils, Odontoblast-Like Cell Macrophages, and Fibroblasts

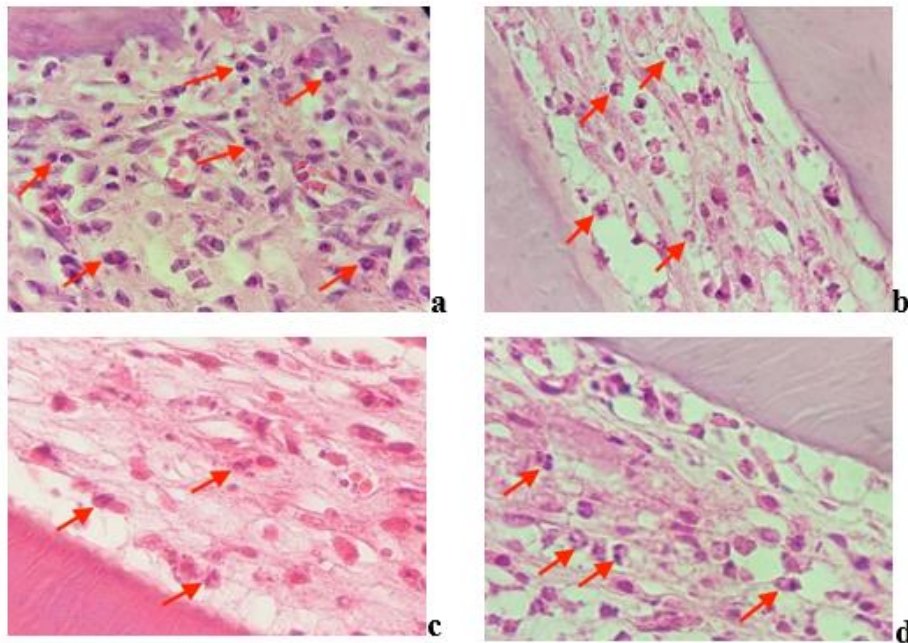
Groups	Control	Ca(OH) <sub>2</sub>	MTA	Biodentine
Control (-)	-	0.037*	0.000*	0.000*
Ca(OH) <sub>2</sub>	0.037*	-	0.022*	0.001*
MTA	0.000*	0.022*	-	0.153
Biodentine	0.000*	0.001*	0.153	-

Neutrophils are distinguished in Figure 1 by their purple granules and multiple nuclei. The negative control group had the greatest quantity of

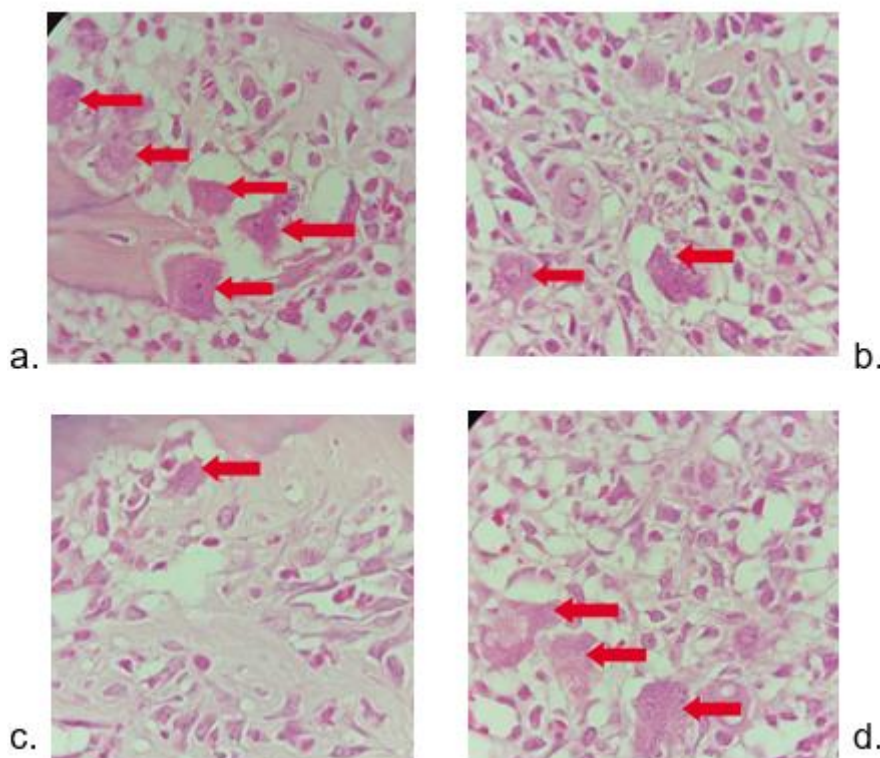
neutrophils compared to the other groups. Figure 2 depicts the identification of macrophage cells that are large, irregular in shape, and have an

eccentrically located kidney-shaped nucleus. The negative control group contained a greater number of macrophages than the other categories. Counts of odontoblast-like cells (Figure 3) and fibroblasts

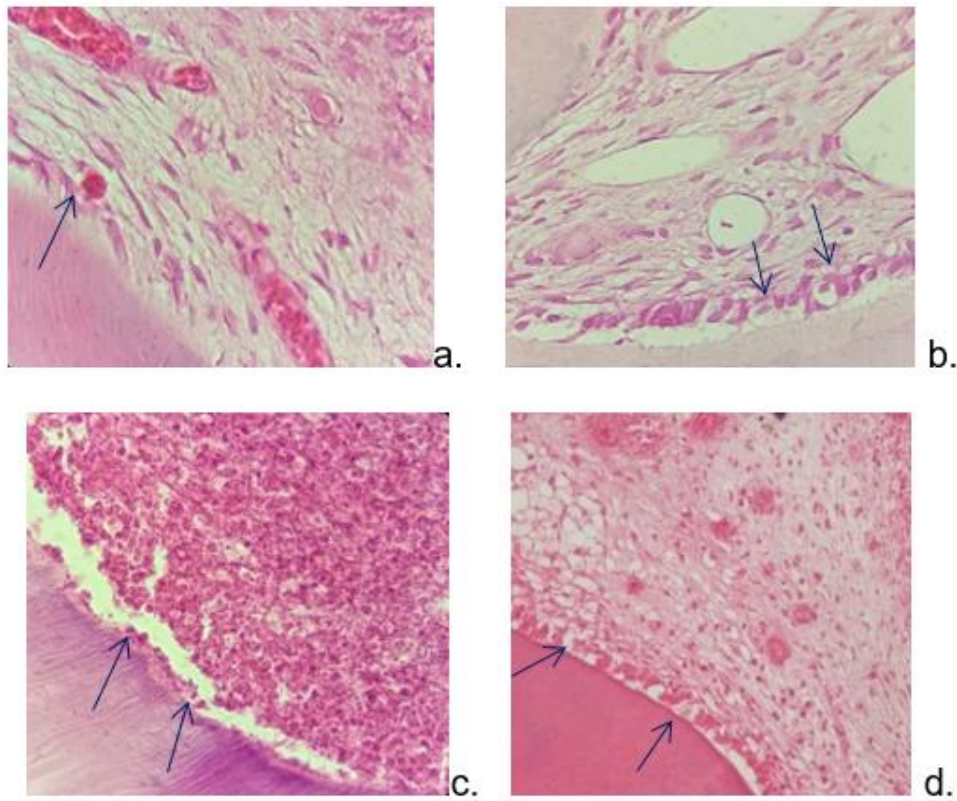
(Figure 4) were greatest in the biodentine, MTA,  $\text{Ca(OH)}_2$ , and negative control groups. Each affirmative cell is indicated by an arrow.



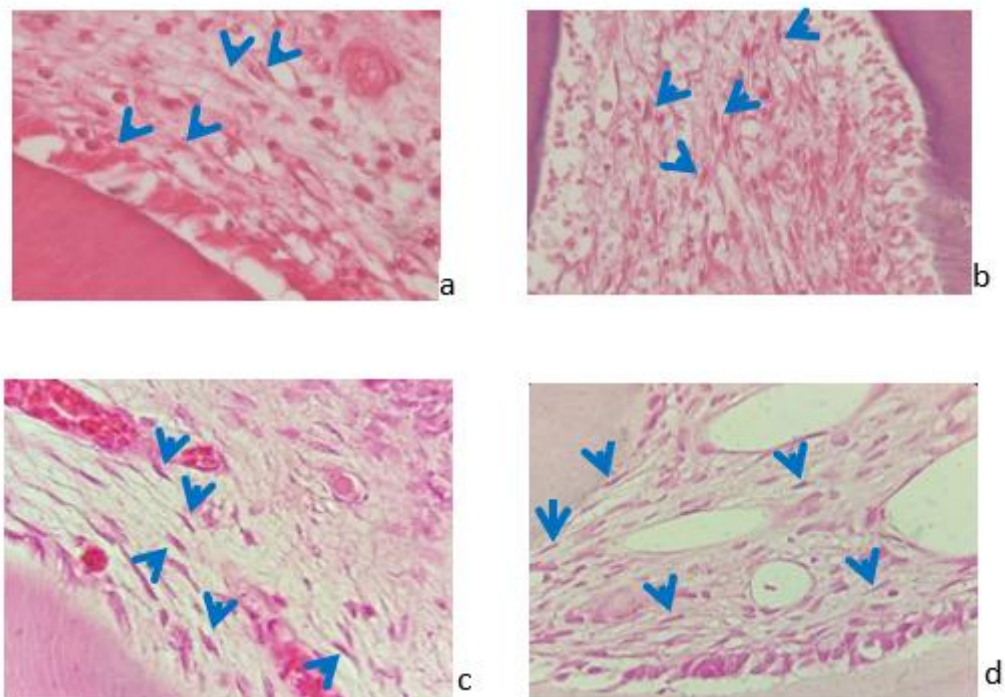
**Figure 1.** Identification of neutrophils by HE staining, at 1000x magnification. (a) Controls; (b) MTA; (c) Biodentine; (d)  $\text{Ca(OH)}_2$



**Figure 2.** Identification of macrophage cells by HE staining, at 1000x magnification. (a) Controls; (b) MTA; (c) Biodentine; (d)  $\text{Ca(OH)}_2$ . Macrophages are seen (red arrow).



**Figure 3.** Identification of Odontoblast-Like Cell with HE staining, at 1000x magnification. (a) Controls; (b) Ca(OH)<sub>2</sub>; (c) MTA; (d) Biodentine.



**Figure 4.** Identification of Fibroblasts by HE staining, at 1000X magnification. (a) Controls, (b) Ca(OH)<sub>2</sub>, (c) MTA, (d) Biodentine. Fibroblasts are seen (blue arrows).

## DISCUSSION

In this investigation, the inflammatory pulpal process was triggered by exposing the pulp roof during tooth preparation with a round bur. Friction and heat induced by the use of rotary instruments during preparation increased nitric oxide levels in odontoblast cells.<sup>13,14</sup> Nitric oxide is a free radical that can induce vasodilation of blood vessels, thereby allowing inflammatory cells, such as neutrophils, to migrate to the tissues.<sup>14,15</sup>

When an injury occurs, the odontoblast is capable of releasing the inflammatory cytokine IL-8, which acts as a motivator for neutrophil migration to the site of injury.<sup>14,16</sup> The low quantity of neutrophils and macrophages in the MTA and biodentine groups may be attributable to the presence of anti-inflammatory calcium silicate. Silica ions can stimulate the proliferation of fibroblasts to produce collagen, followed by a reduction in inflammatory cells such as neutrophils and macrophages<sup>17</sup>. MTA can stimulate neutrophil cells to produce the cytokine IL-1 $\beta$ . The IL-1 $\beta$  can stimulate fibroblasts to produce twice as much collagen.<sup>18</sup>

When the pulp is exposed, fibroblast activity in the cell-rich zone increases. Until new odontoblasts are formed, fibroblasts will migrate to the dentin surface and mature into pre-dentin. These new odontoblast cells are a type of reparative dentinogenesis that will result in the production of collagen fibers.<sup>19</sup> Medications used to treat pulp caps can also increase progenitor cells to stimulate growth factor proteins in regulating cellular processes and the formation of odontoblast-like cells resulting from undifferentiated cells of the dental papilla mesenchyme in response to replace dying odontoblast cells.<sup>20</sup> Ca(OH)<sub>2</sub> and MTA both have potent antibacterial properties, but MTA's firmer bond with dentin allows it to prevent treatment results from leaking. In addition, because

Ca(OH)<sub>2</sub> is highly soluble in oral fluids, it can only solidify in an arid environment.

MTA can form sturdier dentinal bridges than Ca(OH)<sub>2</sub><sup>21</sup>, minimizing the inflammatory response and pulpal necrosis. MTA and biodentine are derived from the same biocompatible and calcium-producing constituent, namely tricalcium silicate. Both of these medications can enhance the modulation of TGF-1 $\beta$  secretion in odontoblast-like cell formation. A study that compared the particle diameters of the two medications revealed that Biodentine had smaller particles than MTA, resulting in a larger surface area during the hydration reaction.<sup>22</sup> The broad hydration reaction surface accelerates the setting time by 12 minutes. Due to the brief setting time, there will be less contact between calcium ions and tissue phosphate. This is due to the adverse effects of long-term biodentine use, which include the formation of large gaps and a thinner structure than MTA in reparative dentine.<sup>23,24</sup> The greatest number of odontoblast-like cells were found in the biodentine group. Silicon ions and calcium ions produced by the biodentine reaction enhance the proliferation of dental pulp stem cells, facilitate cell metabolism in gene expression, and accelerate the processes of cell differentiation, collagen synthesis, and bone mineralization.<sup>17</sup> As a pulp capping medication, biodentine has a number of advantages, including simpler handling, a faster setting time, greater mechanical strength, no tooth discoloration, and low porosity.<sup>25</sup> Based on the findings of this study, biodentine is as effective as MTA in the process of fibroblasts formation; however, biodentine is recommended for use because MTA has several disadvantages, including a long setting time, difficulty in manipulation, and the potential to discolor teeth.<sup>6,18,20</sup>

## CONCLUSION

Biodentine demonstrated efficacy as a pulp capping material, as indicated by a lower number of neutrophils and macrophages, which are inflammatory markers, and a higher number of odontoblast-like cells and fibroblasts compared to the Ca(OH)<sub>2</sub> and MTA group.

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## REFERENCES

- Macpherson, B.R., Oral Histology A Digital Laboratory and Atlas. *University of Kentucky College of medicine*, 2017. Available at: <http://www.uky.edu/~brmacp/oralhist/html/ohtoc.htm>.
- Avery, J.K., Chiego, D.J., Essential of Oral Histology and Embriology, A Clinical Approach, 3rd ed, 2008, Mosby, USA
- Fuks, A. B. 'Vital Pulp Therapy with New Materials for Primary Teeth: New Directions and Treatment Perspectives', *Journal of the American Dental Association*, 2008, 34(7). doi: 10.1016/j.joen.2008.02.031.
- Bogen, G., Kim, J. S. and Bakland, L. K., Direct pulp capping with mineral trioxide aggregate: An observational study, *Journal of the American Dental Association*, 2008, doi: 10.14219/jada.archive.2008.0160.
- Torabinejad, M. & Walton, R.E., *Endodontics*, 2014, In St Louis: Elsevier, pp. 4–24.
- Nowicka, A. and Lipski, M., Tomographic Evaluation of Reparative Dentin Formation after Direct Pulp Capping with Ca ( OH ) 2 , MTA , Biodentine , and Dentin Bonding System in Human Teeth, *Journal of Endodontics*, 2015, pp. 1–7. doi: 10.1016/j.joen.2015.03.017
- Shayegan, A., & Petein, M., The use of beta-tricalcium phosphate , white MTA , white Portland cement and calcium hydroxide for direct pulp capping of primary pig teeth, *Dental Traumatology*, 2009, 413–419. <https://doi.org/10.1111/j.1600-9657.2009.00799.x>
- Ricucci, D., Pashley, D. H., Tay, F. R., & Niu, L. Mechanism of bioactive molecular extraction from mineralized dentin by calcium hydroxide and tricalcium silicate cement. *Dental Materials*, 2017, 1–14. <https://doi.org/10.1016/j.dental.2017.11.010>
- Cengiz, E., & Yilmaz, H. G. (2016). Gallium , and Garnet Laser Irradiation Combined with Resin-based Tricalcium Silicate and Calcium Hydroxide on Direct Pulp Capping: A Randomized Clinical Trial. *Journal of Endodontics*, 42(3), 351–355. <https://doi.org/10.1016/j.joen.2015.11.015>
- Parirokh and Torabinejad, Mineral Trioxide Aggregate: A Comprehensive Literature. *Journal of Endodontics*, 2010, 36(1), 16–27. <https://doi.org/10.1016/j.joen.2009.09.006>
- Laurent, P., Camps, J., & About, I., BiodentineTM induces TGF-β1 release from human pulp cells and early dental pulp mineralization. *International Endodontic Journal*, 2012, <https://doi.org/10.1111/j.1365-2591.2011.01995.x>
- Shinkai, K., Taira, Y., Kawashima, S., Suzuki, S., & Suzuki, M., Histological evaluation of direct pulp capping with all-in-one adhesives in rat teeth. *Dental Materials Journal*, 2017, 36(3), 348–356.
- Feng Mei Yu, Yamaza T, Atsuta I, Danjo A, Yamashita Y, Kido MA, Goto M, Akamine A, Tanaka T., Sequential expression of endothelial nitric oxide synthase, inducible nitric oxide synthase, and nitrotyrosine in odontoblasts and pulp cells during dentin repair after tooth preparation in rat molars. *Cell and Tissue Research*, 2007, 328, 117 – 127.
- Enggardipta, R. A., Haniastuti, T., & Handajani, J., Efek eugenol terhadap jumlah sel inflamasi pada pulpa gigi molar tikus Sprague Dawley. *Majalah Kedokteran Gigi Indonesia*, 2016, 2(2), 66–73.
- Roitt, IM., *Essential immunology 8<sup>th</sup> ed.*, 2002, 233 Jakarta: Widya Medika,
- Levin LG, Rudd A, Bletsa A, Reisner H., Expression of IL-8 by cells of the odontoblast layer in vitro. *Eur J Oral Sci*. 1999, 107(2): 131– 137.
- Peng, W., Liu, W., Zhai, W., Jiang, L., Li, L., Chang, J., *et al.*, Effect of tricalcium silicate on the proliferation and odontogenic differentiation of human dental pulp cells, *J Endod.*, 37:1240-6.
- Cavalcanti, B. N., de Mello Rode, S., França, C. M., & Marques, M. M. (2011). Pulp capping materials exert an effect on the secretion of IL-1β and IL-8 by migrating human neutrophils. *Brazilian Oral Research*, 2011, 25(1), 13–18.
- Ioannidis, K., Mistakidis, I., Beltes, P., & Karagiannis, V., Spectrophotometric analysis of coronal discoloration induced by grey and white MTA. *International Endodontic Journal*, 2013, 46(2), 137–144. <https://doi.org/10.1111/j.1365-2591.2012.02098.x>
- Octiara, E. Dentin Reparatif dan Growth Factor yang Berperan dalam Dentinogenesis

- Reparatif. *Dentika Dental Journal*. 18(3): 294-299. diambil dari: Haniastuti, T., Nunez, P., & Djais, A.A. 2008. The role of transforming growth factor beta in tertiary dentinogenesis. *Dent J (Maj Ked Gigi)*. 2015; 41 (1): 15-20.
21. Mostafa N, Moussa SA. Mineral Trioxide Aggregate ( MTA ) vs Calcium Hydroxide in Direct Pulp Capping – Literature Review. *Online J Dent Oral Heal*. 2018;(October).
  22. Camilleri J., Hydration Mechanisms of Mineral Trioxide Aggregate. *Intern Endod J.*, 2007, 40 (6):462-70
  23. Kaur, M., Singh, H., Dhillon, J. S., Batra, M., & Saini, M. MTA versus Biodentine: Review of Literature with A Comparative Analysis. *J Clin Diagn Res.*, 2017; 11(8), ZG01–ZG05.
  24. Brizuela, C., Ormeno, A., Cabrera, C., Cabezas, R., Silva, C. I., Ramirez, V., & Mercade, M. Direct Pulp Capping with Calcium Hydroxide, Mineral Trioxide Aggregate, and Biodentine in Permanent Young Teeth with Caries: A Randomized Clinical Trial. *J Endod*. 2017; 43(11): 1776-1780.
  25. About, Imad. Biodentine: From Biochemical and Bioactive Properties to Clinical Applications. *Giornale Italiano di Endodonzia*. 2016; 30: 81-88.