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Establishment Differences Odontoblast Like Cell On Reversible Pulpitis With MTA And Nano Hydroxyapatite Sell Duck Eggs (In Vivo Study)

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ABSTRACT

Reversible pulpitis is a pathological condition of the pulp which can return to normal when the etiology of the condition is removed. Reversible pulpitis can be treated with pulp capping treatments to stimulate formation odontoblast like cell. Alternative materials are mineral trioxide aggregate (MTA), Biodentine and Nano hydroxyapatite (nHA) from duck shells. Purpose: to find out whether there is a difference in the number of formations odontoblast like cell in reversible pulpitis with MTA and nano hydroxyapatite duck egg shell. in vivo laboratory experiment test only control design with the independent variables, namely MTA and Nano Hydroxyapatite duck eggs and the dependent variable, namely the difference in the number of formation Odontoblast like-cell. The sample used was the RA right molar tooth of a male Sprague Dawley rat. Results: The results obtained were (p value 0.001), there was a significant difference between the average number of formations odontoblast like cell in each treatment group and control group. Administration of Nano hydroxyapatite medicament from duck egg shells and MTA medicament in the treatment of pulp caps can increase the formation of odontoblast like cell in reversible.

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1. INTRODUCTION

Caries and periodontal disease are one of the most common dental and oral health problems in Indonesian society. The high prevalence of this disease is caused by a lack of knowledge regarding maintenance and awareness of dental and oral hygiene. Data from the Health Research and Development Agency in Riskesdas 2018, the proportion of dental and oral problems is quite high, reaching 57.6%. The number of cases of cavities in individuals aged 5-6 years reached 93%, aged 12 years amounted to 65.5%, aged 15 years amounted to 67.4%, aged 35-44 years amounted to 92.2%, and aged 65 years reached 95%. These data indicate that the rate of dental caries has increased [1].

The pulp as part of the tooth is a soft tissue filled with nerves and blood vessels which functions as a supporting tissue for the tooth structure and the inner part of the dentin that surrounds it. Pulp disease is grouped into several types, namely reversible pulpitis, irreversible pulpitis and pulp necrosis. Pulpitis is inflammation due to a response to a trauma or caries progression in the vascular connective tissue of the pulp [2]. Reversible pulpitis is a pathological condition of the pulp that can return to normal when the etiology of the condition is removed, while irreversible pulpitis cannot return to its original condition even though the etiology has been eliminated [3].

Reversible pulpitis can be treated with pulp capping treatment, which is an endodontic procedure that aims to maintain the vital status of the pulp and can regenerate cells such as sel odontoblast, fibroblas and inflammatory cell infiltrate [4]. Pulp capping actions are classified into two, namely direct pulp capping where treatment is carried out in pulp conditions that are

open due to trauma or operative procedure errors and indirect pulp capping treatment is carried out on pulps that are mildly inflamed but the pulp has not been opened [5].

The injured pulp will produce a defense reaction in the form of collagen synthesis, an inflammatory response and the formation of reparative dentine. Reparative dentine is tertiary dentine which is formed due to strong stimulation and becomes a response by odontoblast starting with formation odontoblast like cell [6]. Odontoblast-like cells is a derivative of odontoblast cells that experience death so they cannot form the extracellular matrix during the formation of dentine bridges produced from a medicament. The presence of injury can encourage the activity of odontoblast cells in increasing expression transforming growth factor beta 1 (TGF-β1) in proliferation mesenchymal cells pulp to carry out mitosis and differentiation to form odontoblast-like cell, The Odontoblast-like cell will mineralize into reparative dentin [7].

In pulp treatment, there are several medicinal ingredients to stimulate formation odontoblast like cell, that is calcium hydroxide as the gold standard because it has high antibacterial properties and can promote the formation and calcification of dentine bridges in exposed pulpal areas. Calcium hydroxide has the disadvantage of causing tunnel defects which creates the potential for bacterial infection and results in liquefaction necrosis in the superficial layers of the pulp. Toxic from calcium hydroxide over time can cause coagulation necrosis at the vital tissue border so that it can mildly irritate the pulp [8].

Alternative materials are mineral trioxide aggregate (MTA), Biodentine and Nano hydroxyapatite. Trioxide aggregate MTA consists of calcium, aluminum and selenium. MTA compared to other medicaments has a smaller risk of inflammation, easier proliferation and differentiation of tooth cells, and the ability to stimulate dentine bridges and hard tissue formation more quickly. The most important properties viz biocompatibility and power capabilities seal. Disadvantages of using MTA are the possibility of releasing harmful substances in the body, long setting time and expensive. Biodentine is a mixture of calcium silicate powder and liquid that can stimulate the formation of tertiary dentine layers [9]. Synthetic hydroxyapatite (HA) is a composition stoichiometry with the chemical formula name Ca10(AFTER4)6(OH)2 on biocompatibility very well with the structure of human bones and teeth [10]. Hydroxyapatite can be found in chicken egg shells, duck egg shells and seashells. Hydroxyapatite has high biocompatibility properties, very suitable for use in the process of repairing damaged bone and tooth tissue. Hydroxyapatite in the pulp capping material can cause inflammation and necrosis of the pulp in certain cases. As an effort to overcome these problems, HA was developed using the method nanotechnology which is named Nano hydroxyapatite (Nano-HA). Molecules found in Nano-HA very similar to the molecules in dentin and enamel [11].

Nano hydroxyapatite (nHA) in duck egg shells is rich in calcium and phosphate compounds, so that it can be an alternative medicament that is biocompatible to surrounding tissues. Calcium phosphate functions to stimulate the differentiation of stem cells into odontoblast-like cells to increase dentin regeneration so that reparative dentine can be formed properly. Duck egg shells also contain 94-97% calcium carbonate which can be used for the synthesis of calcium in the formation of hydroxyapatite crystals. In addition, the result of calcination is the degree of purity calcium oxide (CaO) from duck eggshells was higher than chicken egg shells and quail eggs [12]. Nano-HA and Mineral Trioxide Aggregate (MTA) generates dentin bridges continuously. Nanoparticles are able to increase absorption, increase stability, have good biocompatibility properties to human bones and teeth, and contain molecules that have properties identical to human dentine and enamel. So, materialNano-HAcan be formulated as an alternative direct pulp capping material [13].

This research uses materials Nano hydroxyapatite which can be found in the shells of duck eggs. Nano hydroxyapatite (nHA) and MTA can be used as pulp capping materials for reversible pulpitis [14]. The success of this treatment can be judged by the number of formations odontoblast like-cell in reversible pulpitis with MTA and Nano Hydroxyapatite on

duck eggs [15]. Formation differences odontoblast like-cell in reversible pulpitis with MTA and Nano hydroxyapatite can be investigated using a mouse model Sprague Dawley [16]. Mouses prague dawley was chosen as the animal model because Sprague dawley has a calmer temperament and a softer tooth structure, making it easier to handle [17]. In this in vivo study, drilling of rat teeth will be carried out to induce inflammation, then direct pulp capping treatment will be carried out. Rat teeth will be observed on the 7th, 14th and 28th days because that time is the time needed for formation odontoblast like cell [18]. Based on the description that has been submitted, the purpose of this research is to find out whether there is a difference in the number of formations odontoblast like cell in reversible pulpitis with MTA and nano hydroxyapatite duck egg shell.

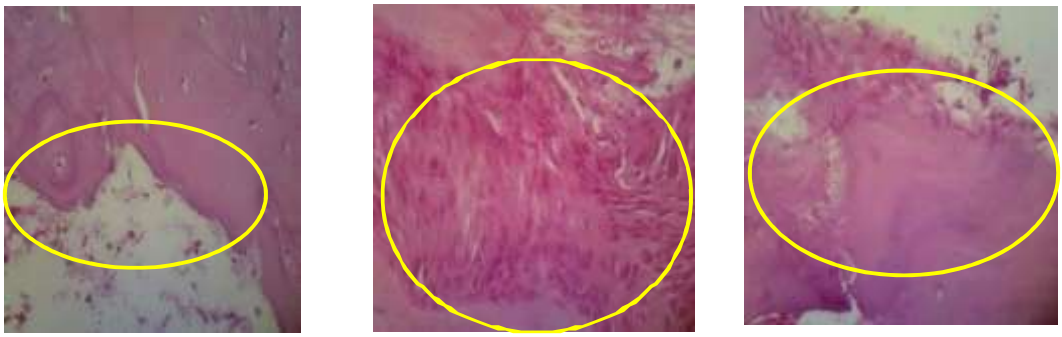
2. METHODS

This research is an in vivo experimental laboratory post-test only control design. The research variables consist of independent variables, namely MTA and Nano Hydroxyapatite duck egg dependent variable, namely the difference in the number of formation Odontoblast like-cell. The population in this study were male Sprague Dawley rats, aged 3 months, weighing 250-300 grams and the sample used was the RA rat's right molar teeth. The study sample size was calculated using the Federer formula with the results. Samples were taken using a simple random sampling technique and divided into 3 groups, namely the control group with reversible pulpitis rats without treatment, the intervention group with reversible pulpitis rats that were treated with reversible pulpitis. Mineral trioxide aggregate (MTA) and other intervention groups which applies nano hydroxyapatite duck egg shell.

The inclusion criteria in this study were male Sprague Dawley rats, 3 months old, 250 – 300 gram body weight, and healthy. While the exclusion criteria were rats that died during the treatment, the temporary fillings fell off, the experimental animals were exposed to a disease. Research done of Pathology and Anatomy Laboratory, Faculty of Medicine, University Muhammadiyah Surakarta. This study used several materials, namely rat treatment materials consisting of Mineral Trioxide Aggregate (MTA), nano hydroxyapatite duck egg, aalcohol 70%, ketamin HCL 0,1 ml/gr BB, saline, glass ionomer cement, Chloroform, and materials for making histological preparations. As well as several tools, namely rat treatment tools, tools for making histological preparations, and observation toolsodontoblast like cell. Data analysis using test One Way Anova because the previous data was normally distributed and homogeneous.

3. RESULTS AND DISCUSSION

Research on formation differences odontoblast like cell on reversible pulpitis with MTA and nano hydroxyapatite of duck egg shells with the Sprague Dawley rat animal model has been completed. The number of odontoblast like cells was counted using a microscope *Optilab Olympus* CX23 and *software image raster*. Calculation *odontoblast like cell* carried out on the 14th day after treatment on Sprague Dawley rats by observing 5 random field of view with 200x magnification with results like the image below:



a *b* c

Figure 1. a). Odontoblast like cell without medicaments (directly given Non Medicament), b). Odontoblast like cell given MTA, c). *Odontoblast like cell* given medicaments nanohidroksiapatit dick egg shells

Table 1. The average yield of Odontoblast like cell

Group	Treatment	Mean ± SD
1	Non Medicament	16,56 ± 7,333
2	MTA	29,56 ± 6,146
3	NHA	25,33 ± 4,899

The results of data analysis show the average formation *odontoblast like cell* the lowest was ingroup 1, namely the rat group that was only given Non Medicament, while the average formation *odontoblast like cell* the highest occurred in group 2, namely the group of rats that were given MTA medicament. Number of forming percentages *odontoblast like cell* from each group parametric analysis using*test One Way Anova*, after fulfilling the requirements, namely the data is normally distributed and thevariance of the data is homogeneous.

Table 2. The Result of *Shapiro Wilk*

Group	Treatment	Sig
1	Non Medicament	0,087
2	MTA	0,138
3	NHA	0,122

Based on the table above, groups 1, 2 and 3 show a significant value of > 0.05 which indicates that the data is normally distributed. Next, a *homogeneity test* was carried out using *Levene Test*. Testresults *Levene Test* can be seen in the table below.

Table 3. Test Results Levene Test

Levene Test	Sig
0,020	0,980

The table above shows that the significant value obtained is 0.980, meaning it is significant > 0.05. So that the data has a homogeneous variant. Then the next test is carried out *One Way Anova*.

Table 4. Test *One Way Anova*

Group	Sig
Non Medicament	
MTA	0,001
NHA	

The table above shows that the significant value obtained is 0.001 (p <0.05), so that there

is a significant difference between the average number of *odontoblast like cell* in each treatment group and control group. Then do the LSD test *(Least Significane Different)* to test the differences between eachgroup.

Table 5. LSD (LEAST Significane Different) test

	1	2	3
1	-	0,001	0,003
2	0,001	-	0,446
3	0,003	0,446	-

Based on the results of the LSD test (*Least Significane Different*) found that there was a significant difference in the number of odontoblast-like cell formation ($p < 0.05$), namely in group 1 togroup 2, group 1 to group 3. While the other groups, namely group 2 to group 3, showed the formation*odontoblast like cell* which is not significant ($p > 0.05$).

Discussion

This study shows the formation *odontoblast like cell* on the teeth of rats with reversible pulpitis which can be seen from the total score*odontoblast like cell* in each group of mice. The greater the number of scores, the greater the number of formations *odontoblast like cell*. On the test *One Way Anova* shows that the significant value is <0.05 so that from the results of these data it can be seen that there are differences in formation*odontoblast like cell* significant difference between the three groups of rats [7]. *Odontoblast like cell* have the same shape as cells *odontoblast*. This *odontoblast* located atthe edge of the pulp tissue formed from ectomesenchymal cells derived from cell migration *neural crest* during early craniofacial development. Cell *odontoblast* differentiated into a columnar form with the nucleus at the proximal pole of the cell body. Cell *odontoblast* It maintains the tooth by forming a new layer of dentine while the pulp is still alive [19].

Trauma to rat teeth caused by blunt use round diamond bur causes the pulp to open, the exposed pulp causes bacteria to enter the rat's teeth resulting in inflammation growing larger into reversible pulpitis and resulting in odontoblast dead. Cell odontoblast the dead will be replaced by odontoblast like cell which can form reparative dentine which functions to protect the pulp from irritants so that inflammation in the pulp can subside. Treatment for reversible pulpitis may include direct pulp capping treatment. Direct pulp capping treatment aims to maintain the exposed pulp to remain vital, there are several medicaments used to stimulate the formation of odontoblast like cell, namely MTA and Nano hydroxyapatite from duck eggshells [4]. The application of pulp capping medicaments will help stimulate an increase in the number of progenitor cells which function to stimulate an increase in transforming growth factor beta 1 (TGF-β1) which is the initial stage for the formation of odontoblast like cells produced from undifferential cells of the dental papilla mesenchyme. Increased TGF-β1 is a defense mechanism when there is an incoming [7].

Effect of pulp capping medicaments on formation odontoblast like cell supported by the results of the LSD test (Least Significane Different) which showed that there was a significant difference between the group of mice with reversible pulpitis that were not given medicaments, the group of mice that were given MTA and the group of mice that were given Nano hydroxyapatite. This shows that the application of pulp capping medicaments has an effect on increasing the amount of formation odontoblast like cell in reversible pulpitis. The results of the LSD test in group 2 and group 3 rats showed that the difference was not too significant between the two, the average number odontoblast like cell in group 2 which was given more MTA material than group 3 which was given nano hydroxyapatite duck egg shells. This is because MTA medicaments have a good capacity in stimulating cell differentiation to form a hard tissue matrix. MTA medicaments can also induce osteogenic cells and stimulate the production of BMP-2 and TGF-β1 from fibroblasts to formodontoblast like cell [7].

MTA and nano hydroxyapatite both produce continuous dentin. However, there is a

difference between dentinal bridge formed by MTA and Nano hydroxyapatite. Dentinal bridge formed by MTA is regular with a dentinal tubule pattern, in contrast to Nano hydroxyapatite where the formation of the dentinal tubule pattern is not visible and there is superficial coagulation necrosis on nano hydroxyapatite [13]. The sealing ability of a pulp capping medicament on the dentine surface has an important role in the formation of a dentinal bridge. MTA has better sealing power than nano hydroxyapatite [20].

The difference in the number of odontoblast like cells that was not too significant between MTA and nano hydroxyapatite could also be influenced by the research procedures carried out, negligence during the research procedures and the skills of the operators [21]. The ability of MTA as an anti- inflammatory agent, antibacterial, stimulates activation growth factor and good sealing ability make MTA able to increase the amount of forming odontoblast like cell. Through the research that has been done, it can be concluded that MTA as a medicament is better at increasing the amount of formation *odontoblast like cell* in reversible pulpitis.

4. CONCLUSION

Administration of *hydroxyapatite Nano* medicament made from duck egg shells in the treatment of pulp caps can increase formation *odontoblast like cell* in reversible pulpitis using rats*Sprague Dawley*. However, amount *odontoblast like cell* produced with the MTA medicament is still more than the *Nano hydroxyapatite* medicament made from duck egg shells. Further research is needed regarding the most effective dose of duck egg shell *nano hydroxyapatite* used as a pulp cap medicament, when isthe peak formation time *odontoblast like cell* on the pulp that is applied with *nano hydroxyapatite* from duck egg shells, and what material is better for making *nano hydroxyapatite* as a medicament ingredient.

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