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Dynamic Level of Fibronectin in Calcium Hydroxide and Mineral Trioxide Aggregate Used as Pulp-Capping Materials

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Abstract

Objectives The aim of this study was to evaluate the dynamics of fibronectin levels in teeth treated with pulp capping using calcium hydroxide ($Ca(OH)_2$) and mineral trioxide aggregate (MTA).

Materials and Methods Test mice were divided into two treatment groups for pulp capping, with group 1 receiving $Ca(OH)_2$ and group 2 receiving MTA. The maxillary first molars of each group's six rats were pulp capped. After pulp capping, blood samples were collected at 1 hour, 24 hours, and 48 hours. A subsequent analysis of the ELISA—enzyme-linked immunosorbent assay—data was performed on additional samples. Hasanuddin University's Faculty of Medicine's Research Ethics Committee allowed the use of experimental animals.

Statistical analysis The data normality test uses the Kolmogorov-Smirnov test (sample $>$ 50) or the Shapiro-Wilk test (sample 50) to identify the analytical application of the distribution of the standard data. Wilk test outcomes had a value of $p > 0.05$; for both the fibronectin and Shapiro leptin levels, the data are considered to be normally distributed.

Results Following the application of $Ca(OH)_2$ and MTA to the treated tooth pulp capping, changes in the levels of leptin and fibronectin were observed after 1 hour, 24 hours, and 48 hours. These changes generally tended to decrease leptin levels and increase fibronectin levels.

Conclusion As a pulp-capping material, the results showed that MTA materials have higher levels of dynamics of fibronectin than $Ca(OH)_2$. This indicates MTA is a better material for pulp-capping treatment.

Keywords
- fibronectin
- pulp capping
- calcium hydroxide
- MTA

Introduction

Fibronectin is a glycoprotein with high molecular weight and is included in the extracellular matrix proteins that bind to membrane receptors, also called integrins.[1] Fibronectin serves as a regulator of cellular processes and also maintains a network organization and composition of the extracellular matrix.[2] Fibronectin is an important glycoprotein that is involved in the wound healing process and dentinogenesis.[3]

The American Association of Endodontists describes "pulp-capping treatment" as the process of applying dental materials such as calcium hydroxide ($Ca(OH)_2$) or mineral trioxide aggregate (MTA) to injured pulp to stimulate the growth of reparative dentin.[4,5]

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$Ca(OH)_2$ and MTA are commonly used to treat pulp capping. These materials are intended to stimulate the dental pulp and form reparative dentin to protect and maintain the vitality of the pulp. Many studies have been carried out to examine the benefits and drawbacks of $Ca(OH)_2$ and MTA used in pulp-capping treatment. Until now, no biomarkers can be used to predict the effectiveness of pulp-capping therapy. Fibronectin is expected later to become one of the markers that can be used to predict the success of pulp-capping treatment. The first step is to investigate the dynamics of fibronectin levels against $Ca(OH)_2$ and MTAs used in pulp-capping treatment.

Materials and Methods

Samples animals eligible for testing were rats that received action on tooth pulp-capping treatment. The inclusion and exclusion criteria were as follows. The inclusion criteria: male gender, age 40 to 60 days, weight 300 to 400 grams, sound and healthy teeth, good health, active, and expected behavior. Exclusion criteria: decreased body weight of rats, rats in an inactive state, and the rats died within the study period. All rats were given the same food and schedule. Unit analysis was done on blood sample taken from a supraorbital vein, based on the length of time the pulp-capping material was applied: 1 hour, 24 hours, 48 hours, and before the application.

In this study, the number of treatments was 2, and the use of test animals was 8 rats equally for each treatment group with four sampling times: 0 hour, 1 hour, 24 hours, and 48 hours; it is based on preliminary tests that have been carried out, as fibronectin expression appears at that time. Total number of test animals in this experimental laboratory study was 16 rats. A sampling of populations with systematic research was conducted by following the working procedures: rats that met the inclusion and exclusion criteria were adopted in the cage in the animal lab for 7 days and given a scheduled meal. Hasanuddin University's Faculty of Medicine's Research Ethics Committee authorized the use of experimental animals. Each animal was given sedation with a mix of ketamine 0.2 mL and xylestesin 0.1 mL to give a calming effect on the animal before dental cavity preparation and blood sampling was done. Before the dental cavity preparation, baseline blood sampling at 0 hour was conducted on supraorbital vein of experimental rats. Rats' mouth were opened and fixed using a mouth guard/retractor to facilitate dental cavity preparation.

The teeth used in this research are the permanent maxillary molars, both right and left. All teeth are scaled using an ultrasonic tool scaler and cleaned using a brush cup before cavity preparation. The isolation quadrant of the teeth is to be prepared using sterile cotton rolls, disinfection done using sodium hypochlorite 2.5%, and saliva controlled using high-speed evacuation. For Class I cavity preparation, a diamond round bur is used with a head diameter of 1 mm (ISO # 806; S.S. White, Lakewood, New Jersey, United States), and a low-speed handpiece with sterile water spray to work on the occlusal surface of the molars. The new and sterile bur is used in every four cavities so the bur is not blunt and to avoid excessive heat on the bur. The cavity is formed having a diameter size of between 0.8 and 1 mm according to the diameter of the bur used. The cavity was cleaned using sterile saline and dried using a paper point. The smallest possible perforation of the cavities into the pulp chamber is made using a sharp straight sonde. Bleeding is controlled by using paper points with light pressure on the open pulp area. In both right and left open maxillary molar tooth pulp, direct pulp capping using MTA or $Ca(OH)_2$ is conducted, and the cavity is restored with type II glass ionomer (Fuji 9, GC Corp, Tokyo, Japan) by following the instructions for the use of the plant. Experimental animals are given sedation to provide a calming effect for the period of supraorbital venous blood sampling, namely at 1 hour, 24 hours, and 48 hours. Also, 1 to 2 mL blood samples are taken using microhematocrit capillary, and blood samples are stored in the sample cup and cooled in a cooler box. The blood sample is sent to a laboratory to examine the levels of fibronectin using the ELISA—enzyme-linked immunosorbent assay—test (Reagent kit Fibronectin Rat [Abcram]). Data processing is analyzed using SPSS 16.0.

Result

The experiment was carried out by following established research methods and use of animal subjects was approved by the Health Research Ethics Committee of Hasanuddin University's Faculty of Medicine.

Following that, the data were processed using a statistical analysis tool. The first step in the analysis of data is the data normality test, which uses the Kolmogorov-Smirnov test (sample > 50) or the Shapiro-Wilk test (sample 50) to ascertain if the distribution of the standard data is analytically utilized or not. Wilk test result from Shapiro leptin levels and fibronectin has a value of $p > 0.05$, so it can be concluded that the data are typically distributed.

Using materials such as $Ca(OH)_2$ and MTA, ►**Fig. 1** illustrates the average changes in fibronectin levels before and after applying pulp-capping materials over the observation times of 1 hour, 24 hours, and 48 hours.

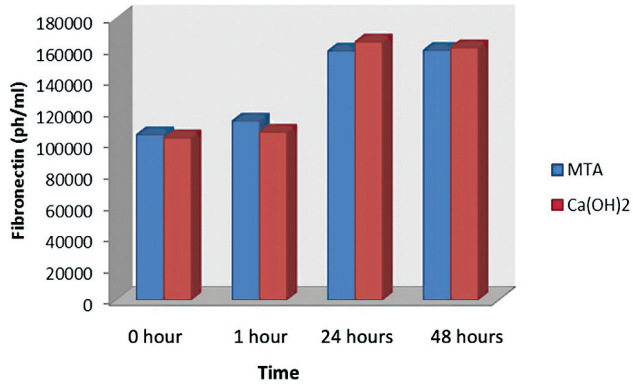

Fig. 1 The average fibronectin concentrations before and after application of the materials for pulp capping. $Ca(OH)_2$, calcium hydroxide; MTA, mineral trioxide aggregate.

Differences in Dynamics of Fibronectin Levels against the Applications of Pulp-Capping Materials Using Ca(OH)2 and MTA

►**Table 1** displays the results of the data analysis of the fluctuations in fibronectin levels after pulp-capping material application using materials Ca(OH)2 and MTA at 1 hour, 24 hours, and 48 hours.

►**Table 1** shows a statistically significant difference in fibronectin dynamics levels between pulp-capping materials $Ca(OH)_2$ and MTA after 1 hour ($p < 0.05$). However, over a longer duration, such as for periods of observation that were extended up to 24 hours and then also further till time reached up to 48 hours, substantially improved outcomes were found when using MTAs. Throughout the observation period starting from 1 hour to 48 hours after applying both materials, it has been observed that overall dynamics levels of fibronectin in MTA are more elevated than those observed in $Ca(OH)_2$. Our observations indicate that levels of dynamic fibronectin were elevated within dental tissue treated with MTA materials relative to $Ca(OH)_2$ treatments, coinciding with earlier research indicating a more significant potential for the induction of reparative dentine.[6,7]

►**Table 2** reveals values of fibronectin dynamics levels; the difference is statistically significant at the beginning of the application of material for pulp capping using $Ca(OH)_2$ and MTA, but the overall level of fibronectin in teeth treated with MTA is higher than when treated with $Ca(OH)_2$.

Discussion

Fibronectin and tenascin are two pieces of noncollagen protein of the extracellular matrix that arise during dentinogenesis. Both of these molecules accumulate in the lining epithelium of the enamel of the teeth basement membrane, and both can trigger odontoblast differentiation.[3,8] At tertiary dentinogenesis, which contains the fibronectin matrix, it can be a reservoir of growth factors, which has been known as a signaling molecule for the differentiation of new odontoblast cells.[8] Fibronectin and tenascin are essential in the dentinal fibromatrix to support cell migration and differentiation. The appearances of fibronectin and tenascin were in line with changes in the matrix, them becoming calcified fibrodentinal bridge, and both are transformed into nonmineralized parts of the dentinal bridge.[9] In conclusion, the present study has indicated that MTA and $Ca(OH)_2$ have the same advantage, which can induce the formation of fibronectin matrix rich in tenascin, which will play a role in cell adhesion and mobilization, as well as trigger differentiation of odontoblasts and form dentinal bridge after pulp-capping treatment. MTA has been shown to effectively stimulate the formation of tertiary dentine and reparative response by inducing the formation of a material such as $Ca(OH)_2$. However, MTA has no content in its composition; there is only calcium

Table 1 Differences in the levels of fibronectin dynamics on the application of pulp capping material using calcium hydroxide ($Ca(OH)_2$) and mineral trioxide aggregate (MTA)

		n	Mean ± SD	*p*-Value
Early fibronectin	MTA	8	162,262.1 ± 35,910.9	0.001[a]
	$Ca(OH)_2$	8	103,388.1 ± 14,754.5	
Fibronectin at 1 hour	MTA	8	113,653.6 ± 32,955.1	0.618
	$Ca(OH)_2$	8	107,055.0 ± 15,921.4	
Fibronectin at 24 hours	MTA	8	196,139.8 ± 39,835.4	0.160
	$Ca(OH)_2$	8	164,617.3 ± 45,051.9	
Fibronectin at 48 hours	MTA	8	163,299.9 ± 23,538.4	0.861
	$Ca(OH)_2$	8	160,901.5 ± 29,909.2	

Abbreviation: SD, standard deviation.
[a]Significant with independent *t*-test.

Table 2 Changes in fibronectin levels before and after pulp-capping treatment using calcium hydroxide observed at 1 hour, 24 hours, and 48 hours

		Mean ± SD			
	n	Before	After	Change (rising)	*p*-Value
Initial fibronectin at 1 hour	8	103,388.1 ± 14,754.5	107,055.0 ± 15,921.4	−3,666.9 ± 18,268.3	0.588
Initial fibronectin at 24 hours	8		164,617.3 ± 45,051.9	−61,229.1 ± 52,999.7	0.014[a]
Initial fibronectin at 48 hours	8		160,901.5 ± 29,909.2	−57,513.4 ± 28,778.5	0.001[a]

Abbreviation: SD, standard deviation.
[a]Significant with independent *t*-test.

oxide, which will form $Ca(OH)_2$ if it binds with water.[6] The high pH levels of MTA will extract growth factors from the nearest dentin and participate in forming a dentinal bridge.[10]

Conclusion

The results showed that fibronectin dynamics levels are higher in MTA when used as pulp-capping material than in $Ca(OH)_2$. This suggests that MTA is a better material for pulp-capping treatment than $Ca(OH)_2$. However, there was no significant difference between 24 and 48 hours. The results need to be further evaluated.

Conflicts of Interest
None declared.

Reference

1 Pankov R, Yamada KM. Fibronectin at a glance. J Cell Sci 2002;115 (Pt 20):3861–3863
2 To WS, Midwood KS. Plasma and cellular fibronectin: distinct and independent functions during tissue repair. Fibrogenesis Tissue Repair 2011;4:21
3 Thesleff I, Vaahtokari A, Partanen AM. Regulation of organogenesis. Common molecular mechanisms regulating the development of teeth and other organs. Int J Dev Biol 1995;39(01):35–50
4 Ingle JI, Bakland LK. Endodontics 4th ed. Philadelphia: Lea and Febiger, 1994
5 Goldberg M. Understanding Dental Caries. Paris, France: Springer; 2016
6 Ford TR, Torabinejad M, Abedi HR, Bakland LK, Kariyawasam SP. Using mineral trioxide aggregate as a pulp-capping material. J Am Dent Assoc 1996;127(10):1491–1494
7 Faraco IM Jr, Holland R. Response of the pulp of dogs to capping with mineral trioxide aggregate or a calcium hydroxide cement. Dent Traumatol 2001;17(04):163–166
8 Yoshiba N, Yoshiba K, Iwaku M, Nakamura H, Ozawa H. A confocal laser scanning microscopic study of the immunofluorescent localization of fibronectin in the odontoblast layer of human teeth. Arch Oral Biol 1994;39(05):395–400
9 Zarrabi MH, Javidi M, Jafarian AH, Joushan B. Immunohistochemical expression of fibronectin and tenascin in human tooth pulp capped with mineral trioxide aggregate and a novel endodontic cement. J Endod 2011;37(12):1613–1618
10 Asgary S, Eghbal MJ, Parirokh M, Ghanavati F, Rahimi H. A comparative study of histologic response to different pulp capping materials and a novel endodontic cement. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;106(04):609–614

