ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

In-Vivo Assessment of Dentin Bridge Formation after Using MTA and Experimental PropolisPaste as Direct Pulp Capping Material.

Reham S.Saleh[1]*, Shaymaa M Nagi[1], Maram E Khallaf[1],Sameh H Abd El-Alim[2], Mohamed H Zaazou[1], Ashraf M Abu-Seida[3], and Mokhtar N Ibrahim[4].

[1]Restorative and Dental Materials Department, Oral and Dental Research division, National Research Centre, Giza, Egypt.
[2]Pharmaceutical Technology Department, National Research Centre, Giza, Egypt.
[3]Surgery, Anesthesiology and Radiology Department, Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt.
[4]Operative Dentistry Department, Faculty of Dentistry, Ain Shams University, Cairo, Egypt.

ABSTRACT

The aim of this study was to evaluate the effect of two different pulp capping materials regarding dentin bridge formation after direct pulp capping for one week and two months. A total of 32 teeth were prepared for this study using two male dogs. Class V cavities were prepared on the buccal surface then exposure was done using a small round bur. Teeth were randomly divided into two main groups according to the applied capping materials (n=16); group I: MTA and group II: experimental Propolis paste. Prepared cavities were finally restored using resin modified glass ionomer. Then these two main groups were further divided into two subgroups (n=8) according to the observation period; one week and two months. Results revealed that no dentin bridge was formed after one week with both tested materials. On the other hand at two months observation period; there was dentin bridge formation in both MTA and Propolis groups and the difference was statistically insignificant. There was no statistically significant difference between both materials at each observation period. Experimental Propolis pulp capping material was able to induce reparative dentin bridge formation after two months. Experimental Propolis paste and MTA are successful direct pulp capping materials regarding dentin bridge formation.

Keywords: MTA, Experimental Propolis, Direct Pulp Capping and Glass Ionomer.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Pulp exposure resulted from caries, trauma or unexpected tooth preparation procedures can produce pain and infection. Dental Pulp capping techniques either direct or indirectare an attempt to preserve pulp vitality and avoid extensive treatments [1,2].

Several pulp capping materials had been used for the dressing of the exposed pulp. Many studies considered calcium hydroxide as the gold standard for dental pulp capping. However, drawbacks were reported by using calcium hydroxide as pulp capping material such as the presence of tunnels defects in the dentin barrier, obliterating the pulp chamber by extensive dentin formation, high solubility and absence of adhesion with tooth structure [3-5].

Mineral Trioxide Aggregate (MTA) is widely used in dentistry; one of its important applications is using it as a pulp capping material. MTA has been proved to be effective in stimulating tertiary dentin formation and produce**s** less pulp inflammation [6, 7].However MTA has several drawbacks as delayed setting time and being expensive [6-9].

Propolis is a natural product collected from trees and shrubs by honeybees. The chemical components present in Propolis are flavonoids, phenolics and different aromatic compounds. Flavonoids possess antioxidant, anti-inflammatory, antibacterial, antiviral and antifungal proprieties. Also Propolis was proved to supports the immune system by promoting phagocytic activities, stimulating cellular immunity and improves healing process [10-12].Hence it is recommended as a natural pulp capping material [11,12].

Thus the aim of this study was to compare the effect of experimentally prepared Propolis paste to MTA as a direct pulp capping materials at different observation periods regarding reparative dentin bridge formation.

MATERIALS AND METHODS

Two different pulp capping materials and one restorative material were used in this study. The materials name, composition, brand name and manufacturers **are** listed in Table 1.

Study design

Thirty two teethin two dogs were included in this study. Teeth were randomly divided into two main groups (n=16) according to the used capping materials; group I: MTA and group II; experimental Propolis paste. In each dog 16 teeth were prepared (4 teeth in each quadrant; one central, one canine and two premolars). Then each main group was further subdivided into two subgroups (n=8) according to the observation periods either; one week ortwo months.

Preparation of experimental Propolis paste

Propolis was obtained in the form of fine brown powder. Extraction of the Flavonoid fraction of Propolis was carried out following Parolia et al, 2010 [11] with some modifications. In a bath sonnicator (Branson 2510 E-DHT, Branson Ultrasonic Corporation, USA) 10 gm of Propolis powder was mixed with 250 ml of 70% ethanol for 30 seconds. Then the suspension was filtered and the residue was submitted to second extraction. This process was repeated twice to allow complete extraction of the Flavonoid which is the desired active ingredient. Under reduced pressure; the collected filtered extracts were fully dried.The dried extract was reducedto a fine powder then the powdered extract was kept inside atight container till usage.

Zinc oxide powder (El Nasr Pharmaceutical Chemicals Co., Egypt) was added to the dried Flovonoid rich Propolis extract to obtain a homogenous powder. In order to have paste form for the experimental Propolis, Polyethylene glycol 400 (LobaChemie, India)was added to the previously prepared Propolis/zinc oxide powder mix, and mixed properly by a mortar and pestle to obtain a homogenous paste. The final paste was kept inside a refrigerator at 8°C in a tight container till usage.

Animal model selection

Two healthy male mongrel dogs with average weight (25kg) and complete set of permanent dentition were selected for this study. Each dog was housed in a separate kennel. Experiment was carried in the animal house at Faculty of Veterinary Medicine, Cairo University, Egypt.

Ethical approval was gained from the National Research Center Research Ethics Committee, Egypt (NO 13-026)and Faculty of Dentistry, Ain Shams University, Egypt.

General anesthesia and dog preparation

After fasting for 12 hours; the dogs were premedicated with atropine sulphate (Atropine Sulphate, ADWIA, Egypt) at a dose of 0.05 mg/kg body weight given s/c and 1 mg/kg body weight XylazineHCl (Xyla-Ject, ADWIA, Egypt) given I.M. General anesthesia was induced by ketamine HCl (Keiran, Eimc, Egypt) at a dose of 5mg/kg body weight injected intravenous using an intravenous cannula. The anesthesia was maintained by 25mg/kg incremental doses of 2.5% solution of thiopental sodium (Thiopental Sodium®, EPICO, Egypt) given I.V [13].The oral cavity of each dog was cleaned using Chlorexidine.

Preparation of Class V cavity

Standard Class V cavities were prepared on the cervical 1/3 of the buccal surface of maxillary and mandibular teeth [6,10]. A modified metal band with a central window was used to standardize the prepared cavities to be 3mm ± 0.5 mesiodistally and 2 mm ± 0.5 occlussogingivally., while the cavity depth was 3 mm ± 1mm. A large size carbide round bur (size 7, MANI, INC, Japan) was used for cavity preparation by low speed micromotor [14-16]. Cavity preparation was carried under copious amount of water [14-16].For pulp exposure;small sterile round bur (size 0.5, MANI, INC, Japan) was used. Hemostasis was achieved with a sterile cotton pellet placed over the exposure site [17-19]. Copious amount of water coolant was used to rinse the prepared cavities. Then the cavities were dried using a sterile cotton pellet.

Application of the capping material

Capping materials were applied for each group as follow:

Mineral Trioxide Aggregate was prepared according to manufacture instructions. One sachet of MTA was mixed with distilled water for 30 seconds on a sterile glass slab using a metal spatula [20]. The mix was applied on the exposure site on the axial wall of the prepared cavities.

Experimental Propolis paste was applied on the exposure site on the axial wall of the prepared cavities using small sterile spoon excavator.

Application of the restorative material

Photac Fil Glass ionomer capsule was mixed for 8 seconds in an amalgamator (RotoMix™, 3M, ESPE) at approximately 4,300 rpm according to manufactures instructions. Then prepared cavities were filled with the glass ionomer restorative material. Light curing was done by using halogen light curing device (Eliper™ 2500,3M, ESPE) with an output 600 mW/cm² for 20 seconds [21,14].

Observation period

Dogs were identified by means of number for the observation periods (one week and two months)[22-24]. All dogs were given intramuscular cefotaxime sodium at a dose of 10 mg/kg and diclofenac sodium at a dose of 1.1mg/kg once/day for 5 days after surgery for pain and infection control [25]. Dogs were followed up and properly evaluated during the whole observation period of the experiments.

Euthanasia of Animals

Dogs were scarified either after one week or two months. Anesthetic overdose using 20 ml of 5% thiopental sodium solution was rapidly injected through the cephalic vein.Then surgical removal of both maxilla and mandible was done.

Histopathological evaluation

After dog scarifying; the teeth and the surrounding bone were resected. Each tooth was placed inside a separate container filled with 10% formalin (Gomhorya Company) solution for fixation. Fixation was carried out for one week and every 48 hours the solution was changed [17].Decalcification using 20% Formic acid and 25% Sodium citrate was applied for four months [19,26]. Then teeth were embedded in paraffin wax to allow buccolingual sectioning of 6 *u*m thickness slabs. Sectioning was done in the middle of the restoration parallel to the main vertical axis of each tooth.The obtained slabs were stained with Hematoxylin-eosin and examined by light microscope for histopathological evaluation. Reparative dentin formation scoring system was described in Table 2.

Statistical analysis

The data was collected and tabulated for statistical analysis. Categorical data were presented by frequency and percentage. Chi square test was used to compare between groups. The significance level was set at $P \leq 0.05$.

RESULTS

Table 3 represents the frequency, percentage and results of Chi- square test for each observation period using MTA.There **was** a statistically significant difference between the two observation periods. Two months observation period revealed statistically significant higher dentin bridge formation than one week observation period.

Table 4 represents the frequency, percentage and results of Chi- square test for each observation periods using **experimental** Propolis. Two months observation period revealed statistically significant higher dentin bridge formation than one week observation period.

Table 5 represents comparison between the two pulp capping materials (MTA and experimental Propolis) at both observation periods regarding dentin bridge formation.

Results revealed that at one week observation period; there was no dentin bridge formation with the two tested pulp capping materials. On the other hand at two months observation period; there was dentin bridge formation in both MTA and Propolis groups and the difference was statistically insignificant.

Table (1): Material name, composition, brand name and manufactures.

Material	Composition	Brand Name	Manufactures
MTA	Powder: mixture of Sio_2, K_2O, Al_2O_3, Na_2O, Fe_2O_3, So_3, CaO, Bi_2O_3 and MgO besides insoluble residues of Cao, Kso4, Naso4 and crystalline silica. Liquid: distilled water	MTA angelus	Londrina-PR-Brazil
Experimental Propolis Paste	Flavonoid rich Propolis (2400 mg), Zinc oxide (600 mg), Polyethyene glycol 400 (1400 mg)	Bee Propolis	Emtenan healthy shop
Photac Fill	Powder: Na, Ca, Al, La, flurosilicate and glass activator. Liquid: monomers, oligomers, copolymer acids (acrylic and metallic acids), camphor-quinone, stabilizers and water.	PhotacFill Quick Aplicap	3M [ESPE] **Dental products. St. Paul, MN, USA**

ISSN: 0975-8585

Table (2): Reparative dentin scoring system.

Scoring	Description
0	Absence of dentin bridge.
1	Presence of dentin bridge.

Table (3): Dentin bridge scoring using MTA as a direct pulp capping material after both observation periods.

MTA	Dentin bridge scores	1 week		2 months		*P*-value
		frequency	%	frequency	%	
Direct pulp capping	absence	8	100	0	0	<0.001*
	presence	0	0	8	100	

**: significant at P ≤ 0.05*

Table (4): Dentin bridge scoring using Propolis as a direct pulp capping material after both observation periods.

Propolis	Dentin bridge scores	1 week		2 months		*P*-value
		frequency	%	frequency	%	
Direct pulp capping	absence	8	100	1	**12.5**	<0.001*
	presence	0	0	7	**87.5**	

**: significant at P ≤ 0.05*

Table (5): Comparison between direct pulp capping materials at both observation periods.

Direct pulp capping	Dentin bridge scores	MTA		Propolis		*P*-value
		frequency	%	Frequency	%	
1 week	absence	8	100	8	100	Not computed**
	presence	0	0	0	0	
2 months	absence	0	0	1	**12.5**	0.102 Ns
	presence	8	100	7	**87.5**	

*Ns: non significant.***:**Not computed because the variables are constant***

DISCUSSION

Preserving tooth vitality is the aim of vital pulp therapy (VPT) by eliminating bacteria from the dentin-pulp complex. There are many treatment modalities for VPT in extensively decayed or traumatized teeth. Proper case selection is mandatory in vital pulp therapy [27-29].

To obtain high success rate in VPT, Asagy et al, 2014[29] and Bavana et al, 2015[18] found that the following criteria should be followed: *i*) uninflamed pulp; *ii*) controlled hemorrhage ; *iii*) a non-toxic capping material and *iv*) the capping material and the applied restoration provide proper hermetic seal [19,30].

Direct pulp capping (DPC) is the selected treatment option when a healthy pulp has been accidentally exposed during operative procedures or trauma. The exposure site must be pinpoint in diameter and the tooth must be asymptomatic [16,28,29,31]. The goal of this treatment is to protect pulp from bacteria and stimulate reparative dentin formation; thus maintain pulp vitality.

In our study, MTA was used as a proved successful direct pulp capping material to compare other experimental material with it regarding dentin bridge formation [6,7].

Propolis is a resinous material from various plants produced by honeybees. It has been used in traditional medicine as an antibacterial and anti-inflammatory agent. Flavonoids is the main constituents of Propolis that ensue different effects like regulating the immune response, and preventing bacterial and fungal growth as mentioned by Sabir et al, 2005[12],Torwane et al, 2013[32]and Jahromi et al, 2014[33]. Thus testing such a natural material (Propolis) in the current study seems to be of value.

To facilitate the study of tissue reaction, dogs were selected in this study because dogs have similar pulpal repair as humans but in short duration [34-35].To follow ethical consideration use and scarifying dogs was as little as possible and a negative control wasn't used.

Regarding reparative dentin formation results; when MTA and Experimental Propoliswere used as a direct pulp capping material after one week of observation, no dentin bridge was formed. This is in accordance with Dammaschke et al, 2010 [15],Sabir et al, 2005[12], Ozorio et al, 2012 [10] and Esmeraldo et al, 2013 [16].This might be attributed to the need of longer period of direct contact of MTA to the pulp tissue in order to promote odontoblastic differentiation and reparative dentin formation [26].

After direct contact for two months, in all samples MTA showed reparative dentin bridge formation which is due to its ability to allow the expression of transcription factors like Runx2. Also MTA can up regulate genes like osteocalcin, alkaline phosphatase, and dentin sialoprotein, which are important odontoblastic genes. These genes in turn promote differentiation of the pulpal cells into odontoblast-like cells which are responsible for dentin bridge formation [8]. For Propolis after two months, Propolis can induce reparative dentin formation. After two months of direct contact with Propolis, dentin bridge was formed in most of the samples, which was found in other studies as both Parolia et al, 2010 [11] and Ahangari et al, 2013 [18] found that after 10 and 15 days respectively Propolis is capapble of inducing dentin bridge formation. This favorable result might be due to the presence of flavonoid component of Propolis which was able to induce reparative dentin bridge from differentiation of odontobast like cells which occurred due to interaction between growth factors (TGF)-*β1* and the extracellar matrix. Propolis was capable of collagen synthesis and induce (TGF)-*β1* [12]. Parolia et al, 2010 [11] found that Propolis can stimulate cell metabolism, circulation, various enzyme systems, and collagen formation which share in the dentin bridge formation. Moreover the presence of vitamin C, provitamin A, B complex, arginine and trace minerals such as zinc, copper, iron as well as flavonoids which promote reparative dentin formation.

In this study the results revealed that there is no statistically significant difference between both MTA and Propolisin term of reparative dentin formation. This was in accordance with Ozorio et al, 2012 [10] andParolia et al, 2010 [11].

CONCLUSIONS

Experimental Propolis direct pulp capping material was able to induce reparative dentin bridge after two months observation period. Both experimental Propolis paste and MTA are considered to be successful direct pulp capping materials regarding dentin bridge formation.

REFERENCES

[1] Al-Hezaimi K, Al-Tayar BA, Bajuaifer YS, Salameh Z, Al-Fouzan K, and Tay FR. J Endo 2011:37,667-672.
[2] Al-Hezaimi K, Salameh Z, Al-Fouzan K, Al Rejaie M and Tay F.R. J Endo 2011: 37, 507-512.
[3] Gala-Garcia A, Teixeira KI, Wykrota FH, Sinisterra R.D and Cortés ME. J Braz Oral Res 2010: 24,8-14.
[4] Park HJ, Lee SK, Park SH, Hong CU, Kim HW, Lee HH, Kim EC and Min KS. J Endo 2008:34,666-670.
[5] Tabarsi B, Parirokh M, Eghbal MG, Haghdoost AA, Torabzadeh H and Asgary S. Int Endod J 2010: 43,565-571.
[6] Nair PN, Duncan HF, Pitt Ford T.R and Luder HU. Int Endo Jo 2008: 41,128-50.
[7] Zarrabi MH, Javidi M, Jafarian AH, and Joushan B. J Endo 2010: 36,1778-1781.
[8] Paranjpe A, Zhang H, and Johnson J.D. J Endo 36:1042-1047; 2010.
[9] Leites AB, Baldissera EZ, Silva AF, Tarquinio S, Botero T, Piva E and Demarco FF. J Oper Dent 2011: 36, 448-456
[10] Ozório JE, Carvalho FO, Oliveira DA, Sousa-Neto MD and Perez DE. J Dent Chil 2012:79-2.
[11] Parolia A, Kundabala M, Rao NN, Acharya SR, Agrawal P, Mohan M and Thomas M. Aust Dent J 2010: 64, 55- 59.
[12] Sabir A, Tabbu C.R, Agustiono P and Sosroseno W. J Oral Sci 2005: 47, 135-138.
[13] Abu-Seida A. Vet Med Int 2015: Volume 2015, Article ID 672643, 4 pages,http://dx.doi. org/10.1155/2015/672643
[14] Benoist FL, Ndiaye FG, Kane AW, Benoist HM, Farge P. Int Dent J 2012: 62,33-39.
[15] Dammaschke T, Sagheri D, Schafer E.J Quint 2010: 41,20-30.
[16] Esmeraldo RM, Carvalho MG , Carvalho RA , Lima R and Costa E,M. Braz J Oral Res 2013:27,417-422.
[17] Accorinte ML, Loguercio AD, Reis A, Bauer JR, Grande RH, Murata SS, Souza V and Holland R. Int Endo J 2009: 42,122-128.
[18] Ahangari Z, Naseri M, and Torkaman A. Cell J 2011: 13,223-228.
[19] Banava S, Fazlyab M, ′Heshmat H, Mojtahedzadeh, and MotahharyP. Iran Endo J 2015:10, 82–88.
[20] Ricketts D. Brit Dent J 2001:191,606 – 610.
[21] Madani ZS, Haddadi A, and Ashraphpour M. Int J Mol Cell Med 2014:3,263-271.
[22] Modena KC, Apayco LC, Atta MT, Costa CA, Hebling J, Sipert CR, Navarro MF, andSantos CF. J appl and Oral Sci 2009:17, 544–554.
[23] Berzins DW, Abey, Wilkie CA, and Roberts HW. J Dent Res 2010:89, 82–86.
[24] Hilton TJ, Ferracane JL Mancl L. J Dent Rest 2013: 92,16–22.
[25] Abu-Seida A M. Asian J Anim Vet Adv 2012: 7, 180-186.
[26] Moazzami F, Ghahramani Y, Tamaddon AM, Nazhavani A.D, and Adl A. Iran Endo J 2014: 9, 50–55.
[27] Hilton TJ. J Oper Dent 2009:34, 615–625.
[28] Ghoddusi J, Forghani M, and Parisay I. Iran Endo J 2014:9,15-22.
[29] Koike T, Polan MA, Izumikawa M, and Saito T. Biomed Res Int J 2014:1,1-8.
[30] Asgary S, Fazlyab M, Sabbagh S, and Eghbal MJ. Iran Endo J 2014:9, 295–300.
[31] Parisay I, Ghoddusi J, and Forghani M. Iran Endo J 2015:10, 6–15.
[32] Torwane NA, Hongal S, Goel P, Chandrashekar BR, Jain M, and Saxena. Euro J Dent 2013:7, 461–468.
[33] Jahromi MZ, Ranjbarian P, and Shiravi S. J Dent Res and Dent Clinical Dent Pros 2014:8,130–133.
[34] El-Ashry S, Abu-Seida A, Al-Boghdady H, El-Batouty K and Abdel-Fattah M. Pak Vet J 2013: 33, 48-52.
[35] El Ashry SH, Abu-Seida AM, Bayoumi AA and Hashem AA. Exp Toxicol Pathol 2016: 68, 181–190