



Histopathological Evaluation of Different Pulp Capping Materials.

Mahmoud Elsayed Fetouh¹, Khalid Mohammed Noaman², Ahmed Ata Abed Elghany³

¹ Assistant lecturer of Operative Dentistry Department, Faculty of Dental Medicine (Assuit-Branch), Al-Azhar University, Egypt.

² Professor of Operative Dentistry Department, Faculty of Dental Medicine, Boys, Cairo Al-Azhar University Egypt.

³ Assistant Professor of Operative Dentistry Department, Faculty of Dental Medicine (Assuit- Branch), Al-Azhar University, Egypt.

Abstract : Objective : This study was conducted to evaluate and compare the histopathological effect of three different pulp capping materials ; Dycal, Rootdent MTA and Biodentine on dogs teeth. Animals and methods: Total of 48 teeth from 4 male mongrel dogs enrolled in this study. Class V cavities with pulp exposures of 1-1.2mm were performed. After bleeding control; the teeth were classified into three main groups (n=16); group I capped with Dycal, group II with Rootdent MTA and group III with Biodentine. Each main group was subdivided into two subgroups (n=8); subgroup T1 investigated after 45 days and subgroup T2 investigated after 90 days. AT T1 and T2; the animals were sacrificed and the teeth were obtained and prepared for microscopic investigation for evaluation of hard tissue bridge formation, degree of inflammation and other histopathologic features. Results: Group II and group III exhibited; better quality and high thickness of the formed dentine bridge, less inflammatory pulpal responses and increased thickness of odontoblast like cell layers more than group I with statistically significant difference. Conclusions: Rootdent MTA and Biodentine showed similar favorable results better than Dycal. The inflammatory pulpal responses decreased by time while the thickness of dentine bridge increased.

Keywords - *Dycal, Rootdent MTA, Biodentine, pulp capping, hard tissue bridge, histopathological.*

I. INTRODUCTION

One of the main goals of restorative dentistry for many years was directed toward the maintenance of a healthy and functional pulp through successful healing of exposures, as the pulp has the potential ability to heal

^[1]. Preservation of pulp vitality is very important process especially in young patients and in teeth with complicated multi canal systems, as the vital pulp tissue contributes to the production of secondary and reparative dentin in response to dentin keeps the dentin moist which maintains its resilience and toughness making the teeth can successfully resist the forces of mastication ^[2]. Pulp injury may occur as a result of excessively deep carious lesion, trauma from teeth preparations or/and other form of various stimulus which may results in the destruction of odontoblastic layer on the periphery of the pulp. Management of pulp injury for long time was considered a hopeless task to save it from breakdown by a conservative pulp-capping or pulpotomy procedure, but within the last 80–90 years ago the pulp healing is indeed possible ^[3].

Vital pulp therapy is indicated mainly in those teeth with traumatic or accidental exposure regardless of open or closed apex; which involve removal of local irritants and placement of a protective material directly or indirectly over the pulp to shield the teeth from bacterial intrusion and protect the pulp against thermal and or mechanical stimulus ^[4]. The regeneration of pulp occurs through the recruitment of progenitor cells, their differentiation into secreting cells and hence stimulation of reparative dentinogenesis ^[5]. The first pulp capping procedure was performed in 1756, by Phillip Pfaff, who packed a small piece of gold over an exposed vital pulp to promote healing ^[6]. However Favorable outcome of pulp capping greatly depends on various factors including age, periodontal condition, stage of root formation, size of exposure and its nature, good knowledge of pulp anatomy, capping material and using sterile technique ^[7]. The major advances in the practice of pulp capping materials have been made resulting in a number of materials that have been used as a pulp capping agents, the most prominent of them are calcium hydroxide Ca (OH)₂, Mineral Trioxide Aggregate MTA, and Biodentin ^[8].

For many decades since 1928 calcium hydroxide has been considered the ‘gold standard’ of pulp capping materials because of their property of stimulating sclerotic and reparative dentin formation, protecting the pulp against thermal stimuli and its antibacterial action. It serves as protective barrier for pulpal tissues not only by blocking patent dentinal tubules but also by neutralizing the attack of inorganic acids and their leached products from certain cements and filling materials ^[9]. But it has some drawbacks such as, poor bonding, resorption and mechanical instability, microleakage in the long term. Moreover, the newly formed dentin bridges may contain defects and could act as a portal of entry for microorganisms that might eventually affect the treatment ^[10].

In the past twenty years since 1990 a great attention was given to Mineral Trioxide Aggregate (MTA) as the material of choice for all dentinal defects due to its biocompatibility and high pH value of the material. It produces more dentinal bridging with superior structural integrity in a shorter time span with significantly lesser inflammation, has superior ability to resist for further penetration of bacteria, significant antimicrobial property, hydrophilic as it sets in presence of moisture with little impact on degree of leakage ^[11]. However, MTA has certain drawbacks such as, it is difficult to handle, long setting time, high material cost and potential discoloration of dental tissues in gray material ^[12].

To overcome these deficiencies, the researches were directed toward a new material known as dentin in capsule which became commercially available in 2009 as a Biodentine. It is calcium silicate based restorative cement with dentin-like mechanical properties, excellent biocompatibility, bioactive behavior and good sealing ability ^[13]. It was considered a suitable material for clinical indications of pulp capping as it promotes growth, proliferation and differentiation and consequently the formation of reparative dentin ^[14]. It also exhibit good handling properties, high viscosity, shorter setting time (12 minutes), and better physical properties ^[15-16].

II SUBJECTS AND METHODS

1. Study design: Randomized experimental study.

2. **Study setting:** This study was carried out in the department of surgery, anesthesiology and radiology, faculty of veterinary medicine, El- Saddat University.
3. **Ethical approval:** All international and institutional guidelines for animal use and care were followed up. The protocol of this study was approved by the Ethical Committee of Faculty of Dentistry Al- Azher University (Cairo- Boys).
4. **Animals:** Total of four male healthy mongrel dogs with approximately age of 18–24 months and weight 15–20 kg were selected for this study. The dogs were kept individually in separate cages for two weeks before being included in the study. All the dogs were monitored daily to exclude and evaluate any pathological condition under supervision of an expert veterinarian. They were kept under good conditions of ventilation, cleaning and nutrition of three times a day of cooked or and dry food, pure water was available all the time ^[17].
5. **Classification of the teeth:** Total number of 48 teeth including; lateral incisors, canines, premolars and molars (12 teeth / dog) in three quadrants of each dog were included in this study. The teeth were classified into three equal groups (n= 16) according to the pulp capping materials including: Group I: Dycal, Group II: Rootdent MTA and Group III: Biodentine. Each group was further subdivided into two equal subgroups (n=8) according to the post treatment evaluation periods including; Subgroup T1; investigated after (45 days) and Subgroup T2; investigated after (90 days).
6. **Operative procedures:** Firstly, the experiment was performed in two dogs and after 45 days the other two dogs were included in the study to sacrifice all of them after 90 days. The dogs were premedicated with Atropine sulphate at 0.02–0.04 mg/kg injected subcutaneously. The general anesthesia was routed intravenously with xylazine, ketamine HCL at 6-10 mg/kg and maintained by thiopental sodium at a dose of 25 mg/kg injected through the cannula that fixed in the cephalic vein of the dogs. All doses of premedicaments and anesthetic agents were determined and injected by veterinary anesthesiologist ^[18]. After the anesthesia was achieved; rubber dam was applied and the isolated teeth were cleaned with air/water irrigation, air dried and disinfected with cotton pellet saturated with hexitol solution ^[19]. Class V cavities with approximately 3-5 mm width, 3 mm length and 2 mm depth were made on the buccal surfaces 1mm above the gingival margin ^[20-21] by sterilized carbide burs mounted in high speed handpiece under air/water spray coolant. Pulp exposures were performed by drilling the center of class V cavities floor till bur dropping and the pulp was reached using sharp round burs with approximate head diameter of 1 mm so the size of exposure point was about 1 - 1.2 mm ^[10]. The bleeding was controlled by dressing the exposure site with cotton pellet moistened with saline for about 1 minute until physiologic homeostasis was achieved ^[22]. After homeostasis the exposed pulps were capped with one of the pulp capping material according to the tested groups; Group I: capped with Dycal as control group, Group II: capped with Rootdent MTA and Group III: capped with Biodentine. The materials were mixed and applied over the exposure sites according to the manufacturer's instructions. After setting of the pulp capping materials; thin layer of resin modified glass ionomer was applied and the final restoration was performed by packable composite (P60) which applied and finished according to the manufacturer's instructions ^[23]. At the end of the operation; all dogs were given once daily for five days cefotaxime at a dose of 10 mg/kg IM injection for infection control, and diclofenac sodium at a dose of 1.1mg/kg IM injection for pain control ^[24].
7. **Histopathological evaluation:** After 90 days the four dogs were sacrificed according to the first and second post treatment evaluation periods T1 and T2 using over dose of general anesthetic solution (20 mL Thiopental sodium 5% solution) injected through the cephalic vein. The dogs Jaws were separated and teeth bone blocks were cut by high speed surgical bur, vital perfusion fixation was performed with 10% buffered formalin solution for 2 weeks then rinsed under running tap water for several hours. The specimens were then demineralized in 20% EDTA solution for about 150. Perforation test was carried out by a fine needle with sharp tip to check the degree of decalcification. Decalcification is completed when the hard tissues bone and teeth became soft and pliable. After decalcification, the specimens were rinsed under running tap water for several hours followed by dehydration in ascending concentrations of ethyl alcohol (70%, 90%, and 100%) then embedded in paraffin wax and six micrometer bucco lingual sections were prepared by manual rotary microtome passing through the cavity at the exposure site and stained with Hematoxylin and Eosin (Bancroft 2008) ^[25]. The specimens were

evaluated by an experienced pathologist who was not aware of the types of pulp capping materials. Coded specimens were used throughout the study to avoid possible bias. The stained sections were assessed by the image analysis software. Photomicrographs were captured by Leica EC3 digital camera attached to the light microscope by a C – mount with magnifications ranging from (x40, x100 and x200). The histological changes of the pulp tissues in response to the pulp capping materials were assessed including; Hard tissue formation at the interface of the pulp capping materials with the pulp tissues (continuity, morphology and thickness), degree of pulpal inflammation (type, intensity, and extension), and other histologic features including; (calcifications, necrosis and odontoblast cell layers). The histopathological finding were graded from I to III indicating; grade I (the worst), grade II (intermediate) and grade III (the best) ^[26].

8. **Statistical analysis;** The data were analyzed with Kruskal-Wallis and Mann-Whitney tests. Statistically significant differences were set at $P < 0.05$.

III RESULTS

The results obtained from this in vivo study at both evaluation periods (T1 and T2) as illustrated in TABLES (1,2) revealed that; Regarding the hard tissue bridge formation; most of the specimens capped with Rootdent MTA and Biodentine exhibited uniform, better quality and high thickness of the formed hard tissue bridge which completely or partially closing the exposure site more than that exhibited by specimens capped with Dycal with statistically significant difference. Regarding the inflammatory pulpal responses; most of the specimens capped with Rootdent MTA and Biodentine exhibited no or mild chronic inflammatory pulpal responses and absence of acute inflammation more than that exhibited by specimens capped with Dycal with statistically significant difference. Regarding the other histologic features; most of the specimens capped with Rootdent MTA and Biodentine exhibited increased thickness of odontoblast like cell layers which arranged in palisading pattern with their cytoplasmic processes associated with newly formed dentine bridge, absence of partial or/and complete necrosis and areas of dystrophic calcification associated with the pulp tissues more than that exhibited by the specimens capped with Dycal with statistically significant difference. The difference between Rootdent MTA and Biodentine in all histopathological finding of the pulp tissues was statistically non-significant.

Table (1): Number (%) of different categories of the histopathological finding for each group according to the scores received during histopathological evaluations after 45 days T1 (n = 8 for each group).

Features	Categories	Dycal			Rootdent MTA			Biodentine		
		I	II	III	I	II	III	I	II	III
Hard tissue (dentinal bridge)	Continuity	3 (37.5%)	4 (50%)	1 (12.5%)	1 (12.5%)	3 (37.5%)	4 (50%)	0 (0%)	2 (25%)	6 (75%)
	Morphology	5 (62.5%)	2 (25%)	1 (12.5%)	1 (12.5%)	3 (37.5%)	4 (50%)	0 (0%)	4 (50%)	4 (50%)
	Thickness	6 (75%)	2 (25%)	0 (0%)	2 (25%)	4 (50%)	2 (25%)	1 (12.5%)	3 (37.5%)	4 (50%)
Pulp inflammation	Type	2 (25%)	5 (62.5%)	1 (12.5%)	0 (0%)	2 (25%)	6 (75%)	0 (0%)	2 (25%)	6 (75%)
	Intensity	3 (37.5%)	4 (50%)	1 (12.5%)	0 (0%)	1 (12.5%)	7 (87.5%)	0 (0%)	2 (25%)	6 (75%)
	Extension	1 (12.5%)	6 (75%)	1 (12.5%)	0 (0%)	1 (12.5%)	7 (87.5%)	0 (0%)	2 (25%)	6 (75%)
Other histologic features	Calcification	0 (0%)	5 (62.5%)	3 (37.5%)	0 (0%)	0 (0%)	8 (100%)	0 (0%)	1 (12.5%)	7 (87.5%)
	Necrosis	1 (12.5%)	3 (37.5%)	4 (50%)	0 (0%)	0 (0%)	8 (100%)	0 (0%)	0 (0%)	8 (100%)
	Odontoblastic layers	7 (87.5%)	1 (12.5%)	0 (0%)	0 (0%)	2 (25%)	6 (75%)	0 (0%)	2 (25%)	6 (75%)

Table (2): Number (%) of different categories of the histopathological finding for each group according to the scores received during histopathological evaluations after 90 days T1 (n = 8 for each group).

Features	Category	Dycal			Rootdent MTA			Biodentine		
		I	II	III	I	II	III	I	II	III
Hard tissue (dental bridge)	Continuity	1 (12.5%)	3 (37.5%)	4 (50%)	0 (0%)	1 (12.5%)	7 (87.5%)	0 (0%)	0 (0%)	8 (100%)
	Morphology	2 (25%)	2 (2.5%)	4 (50%)	0 (0%)	2 (25%)	6 (75%)	0 (0%)	1 (12.5%)	7 (87.5%)
	Thickness	3 (37.5%)	4 (50%)	1 (12.5%)	1 (25%)	3 (37.5%)	4 (50%)	0 (0%)	2 (25%)	6 (75%)
Pulp inflammation	Type	2 (25%)	4 (50%)	2 (25%)	0 (0%)	1 (12.5%)	7 (87.5%)	0 (0%)	2 (25%)	6 (75%)
	Intensity	2 (25%)	4 (50%)	2 (25%)	0 (0%)	1 (12.5%)	7 (87.5%)	0 (0%)	2 (25%)	6 (75%)
	Extension	1 (12.5%)	5 (62.5%)	2 (25%)	0 (0%)	1 (12.5%)	7 (87.5%)	0 (0%)	2 (25%)	6 (75%)
Other histologic features	Calcification	0 (0%)	3 (37.5%)	5 (62.5%)	0 (0%)	0 (0%)	8 (100%)	0 (0%)	1 (12.5%)	7 (87.5%)
	Necrosis	1 (12.5%)	2 (25%)	5 (62.5%)	0 (0%)	0 (0%)	8 (100%)	0 (0%)	0 (0%)	8 (100%)
	Odontoblastic layer	2 (25%)	3 (37.5%)	3 (37.5%)	0 (0%)	1 (12.5%)	7 (87.5%)	0 (0%)	0 (0%)	8 (75%)

IV. DISCUSSION

Direct pulp capping is an alternative procedure to extraction or endodontic therapy, which involves the placement of a biocompatible agent on pulp tissue that has been inadvertently exposed from traumatic injury or by iatrogenic means. The aim of the treatment is to maintain healthy pulp tissue by sealing the pulp against bacterial penetration and initiating a dentine bridge formation at the exposure site [27]. Long-term clinical studies indicated that; direct pulp capping can produce success rates of between 80% and 90%. These figures are comparable to the success rate of root canal treatment which is expected to be 85% to 90%. The first pulp capping procedure was performed in 1756, by Phillip Pfaff, who packed a small piece of gold over an exposed vital pulp to promote healing [28].

Clinical and histological evaluations are the gold standard for the assessment of pulp reactions to the tested materials [29]. Both animal and human teeth are suitable to demonstrate the histopathological effects of pulp capping materials on vital pulp tissues. Dogs were the selected model for the current study because the mechanism of induction and synthesis of dentin in this animal are the same as in human beings, even though; the rate of reparative dentinogenesis may differ. Dog dentition includes four premolars and two or three molars in each quadrant which provides a good number of teeth allowing the comparison of more than one material in the same dog [30]. Under general anesthesia; the selected teeth were isolated with rubber dam and disinfected with chlorohixidine as preventing microorganisms from entering the pulp and control of bleeding are the key factors for successful direct pulp capping [31].

Class V cavities with diminutions 3-5 mm width, 3 mm length and 2 mm depth were made on the buccal surfaces 0.5-1mm above the gingival margin. Pulp exposures of approximately 1-1.2 mm were made at the center of the pulpal floor as present in previous studies [32,33]. Complete hemostasis was achieved by applying pressure with cotton pellet moistened with sterile saline. According to the other studies [20,34] Sodium hypochlorite in concentrations of 0.25% was efficacious for hemorrhage control without being toxic. However, since sodium hypochlorite has a pH of 12, it may extract growth factors from the walls of dentine just as $\text{Ca}(\text{OH})_2$. Thus, in the present study, saline was used to control hemorrhage to prevent the supplementary reparative dentinogenesis action of sodium hypochlorite. The exposed pulps were capped with one of the pulp capping materials according to the experimented groups which were mixed and applied over the exposure sites according to the manufacturer's instructions followed by thin protective layer of resin modified glass ionomer base to protect the underlying pulp capping materials from acid etching and contraction stress delivered during polymerization of resin composite [1]. The final restoration was performed with packable composite which applied and cured according to the manufacturer's instructions. The dogs were sacrificed after 45 and 90 days according to the first and second post treatment evaluation periods T1 and T2. Similar to the previous studies [35,36], period of 45 days was chosen to evaluate the effect of capping materials on pulp tissues. In the study performed by Yoshida et al [37], they reported that; after pulp capping with $\text{Ca}(\text{OH})_2$, the appearance of dentine like tissue was observed after 4 weeks. Other studies [38,39], showed that extracts of tricalcium silicate were able to induce human dental pulp stem cells proliferation, differentiation, and mineralization at a faster rate than $\text{Ca}(\text{OH})_2$. Hence, a period of 45 days was justifiable to study the dentine bridge formation histologically.

The results of this study showed that; more favorable histologic results following pulp capping with either Rootdent MTA or Biodentine in comparison to Dycal. In the present study, all pulp capping materials induced the formation of a dentinal bridge at its interface with the pulp tissues which may be partially or completely closed the exposure site. The mechanism of dentine bridge formation varies in the various formulations of $\text{Ca}(\text{OH})_2$ depending on the pH of the material. In the case of a high pH material (pH=12), such as Rootdent MTA and Biodentine a necrotic zone is formed adjacent to the material and the dentine bridge forms between this layer and the underlying

vital pulp, then the necrotic tissue eventually degenerates and disappears leaving a voids between the capping material and the bridge. In the case of a material of lower pH (pH=10) such as Dycal, a necrotic zone is formed adjacent to the material but is resorbed before the formation of the dentine bridge, which then comes to be formed directly against the capping material ^[40].

At both evaluation periods; the pulps that were treated with Rootdent MTA and Biodentine were essentially associated with the formation of a good quality of hard tissue bridge which was more predictable, uniform, thick, continuous, and partially or completely sealed the pulp tissues more than that performed by pulps treated with Dycal. The difference between Dycal and each of Rootdent MTA and Biodentine was statistically significant while the difference between Rootdent MTA and Biodentine was statistically non-significant. This finding in accordance with the studies performed by Ali Eskandarizadeh et al ^[38], Moustafa Mohammed et al ^[15], I.M. Faraco et al ^[20] and Ali Akhavan et al ^[18]. They reported that; the hard tissue bridge performed by teeth capped with MTA and Biodentine is significantly in better quality and high thickness more than that performed by teeth capped with Dycal, inflammatory pulpal responses in teeth capped with MTA and Biodentine is significantly lower than that performed by teeth capped with Dycal and non-significant difference between MTA and Biodentine. They explain their finding to the ability of Dycal to promote the formation of the hard tissue bridge is attributed to induction and upregulation of odontoblast like cell differentiation for new matrix deposition through its solubilizing effect of growth factors from the dentin matrix due to its alkaline pH. Its caustic effect completely deranged and distorted the pulp tissue in immediate contact with Ca (OH)₂, producing a mummified zone, which stimulated the subjacent vital pulp tissue to respond with all its healing potential to produce a dentine bridge. MTA induced cytological and functional changes in the pulp cells leading to the formation of fibrodentine and reparative dentin. MTA contains calcium oxide, which forms calcium hydroxide when mixed with water, the reaction of calcium hydroxide and the carbon dioxide from pulp tissue produced calcite crystals. These calcite crystals attract the fibronectin which is the initiating step in the formation of hard tissue barrier through enhancing cellular adhesion and differentiation. The reparative structures induced by Biodentine were homogenous and in continuity with primary dentine, this better quality of dentinal bridge might be attributed to release of transforming growth factor B1 (TGF-B1) from pulp cells which stimulate the odontoblasts to increase their activity and enhancing reparative dentinogenesis.

In terms of the continuity of the hard tissue bridge, these findings are in agreement with study performed by X. Tran et al ^[39], they reported that; the reparative structures induced by both calcium silicate cements (MTA and Biodentine) were homogenous and in continuity with primary dentine. In contrast, the reparative tissue induced by Ca (OH)₂ had a porous organization this porosity may eventually facilitate bacterial ingress into the pulp, compromising the tooth vitality. In terms of increased thickness of the dentine bridge these findings are in agreement with the study performed by Peng et al ^[41], they reported that; the thickness of hard tissue bridge performed by teeth capped with MTA and Biodentine is more than that performed by teeth capped with Ca (OH)₂. They attributed their finding to that; the extracts of tricalcium silicate (silicon ions) released from silicate containing cements (MTA and Biodentine) enhanced the dental pulp stem cells proliferation, differentiation, and mineralization in comparison to Ca (OH)₂, by involvement in metabolism, collagen synthesis, bone mineralization and connective tissue cross-linking. Also in agreement with the study performed by Marijana Popovic Bajic et al ^[33], they reported that no significant difference between MTA and Biodentine in regard to presence of dentin bridge, an inflammatory reaction of the pulp, odontoblastic cell layers and necrosis.

The results of this study in dis accordance with the study performed by Sepideh Banava et al ^[42], they reported that no significant difference between teeth specimens capped with Dycal and that capped with MTA in regard to formation of hard tissue bridge, but in accordance with this study in

regard to the inflammatory pulpal responses which were significantly lower in specimens with MTA than that of specimens with Dycal. The difference may be due to; in the study performed by Sepideh Banava et al, they used human teeth and pulp exposure of 0.5 mm diameter was performed through class I cavity preparation while in this study we used dog's teeth and pulp exposure of 1-1.2 mm diameter was performed through class V cavity. And in dis agreement with the study performed by Anushka Lalit Jalan et al ^[10], they reported that no statistically significant difference between the Dycal and Biodentine samples in relation to the presence and morphology of the formed dentinal bridge at the pulp medicament interface. The difference may be due to; the pulp exposure which was performed through class I cavity preparation in human teeth. Also in dis accordance with the study performed by M.L. Accorinte et al ^[43], they reported that no statistically significant difference between the Ca (OH)₂ and MTA samples in relation to the formed dentinal bridge at the pulp medicament interface. The difference may be due to different materials and techniques used in the study performed by M.L. Accorinte et al, as they used Ca (OH)₂ powder in human teeth and pulp exposure was performed through class I cavity while in this study we used Dycal in dog's teeth and pulp exposure was performed through class V cavity.

The decreased inflammatory pulpal responses and absence of necrosis in specimens capped with Rootdent MTA and Biodentine more than that capped with Dycal in accordance with the study performed by Nowicka et al ^[44], They attributed their finding to biocompatibility and excellent sealing properties of both materials which prevent microleakage and subsequent pulpal inflammation by providing a predictable secondary barrier under the surface seal, while the increased inflammatory pulpal responses in specimens with Dycal may be due to the microleakage resulting from difficult in sealing ability of the chemically set material in wet environment of the exposed pulp, gradual degradation of the material and the formed secondary hard tissue barrier is thin and porous which may permit passage of bacteria and other irritant to the pulp.

But in dis accordance with the study performed by Fonseca, et al ^[45], they found that the number of inflammatory cells was significantly higher in the Biodentine group in comparison with the MTA in the initial periods. The difference may be attributed to; in the Fonseca study; the experiment was carried out on rats by putting a polyethylene tube filled with experimented materials into the dorsal subcutaneous tissues of rats. Also in dis accordance with the study performed by Tabarsi et al ^[46], they reported that; after direct capping with MTA necrosis was present in 22.7% of samples. Different findings of this study can be explained by the fact that in the study of Tabarsi et al, MTA was placed after pulpotomy while in the current study only small exposed surface of the pulp was covered with MTA.

In most specimens of MTA and Biodentine groups, odontoblasts were arranged just below the dentinal bridge with some structural changes. These cells were not true odontoblasts, but odontoblast like cells having elongated shape and palisade orientation. These findings are in accordance with other previous studies; Eskandarizadeh A et al ^[38] and Saeed A et al ^[47], they reported that, Odontoblast like cells produce extracellular matrix that becomes complete dentine bridge after mineralization. The thickness of dentine bridge and pulp preservation depends upon the amount of odontoblast like cells, with increased layers of these cells, the thickness of dentinal bridge increase and the pulp remains vital, this can explain the increased in thickness and palisading arrangement in odontoblastic cell layers in specimens of MTA and Biodentine groups than that of Dycal group.

V. CONCLUSION

Under the limitations of this study, the following can be concluded;

1. Rootdent MTA and Biodentine showed similar favorable results better than Dycal with statistically significant difference in terms of hard tissue bridge formation, inflammatory pulpal

responses and necrosis when they used as a direct pulp capping materials in traumatically exposed pulp of dog's teeth.

2. Inflammatory pulpal responses decreased by time while the thickness of hard tissue bridge increased.

REFERENCES

- [1] A. Parolia, M. Kundabala, N. Rao, S. Acharya, P. Agrawal, M. Mohan, A comparative Histological Analysis Of Human Pulp Following Direct Pulp Capping With Propolis, Mineral Trioxide Aggregate And Dycal, *Australian Dental Journal*, 55(3), 2010, 59–64.
- [2] D. Witherspoon, J. Small, G. Harris, Mineral trioxide aggregate pulpotomies: a case series outcomes assessment, *Journal of American Dental Association*, 37(2), 2006, 610- 18.
- [3] V. Stefanova, S. Tsanova, P. Atanasova, I. Borisov, T. Chaprazov, M. Tsanova, Early results after direct pulp capping with tricalcium silicate based material and mineral trioxide aggregate and ER: YAG cavity preparation in dogs, *Trakia Journal of Sciences*, 12(1), 2014, 331- 34.
- [4] D. Pooja, S. Yogesh, J. Aditi, M. Praveen, R. Owais, S. Shruti, Biodentine: the new bioactive and biocompatible material of choice for direct pulp capping & Pulpotomy in curiously exposed permanent teeth: case reports, *International Journal of Applied Dental Science*, 4(4) 2018, 09-12.
- [5] G. Anshu , Sohez M, Kiran V, Hitesh S, Kailash A, Rimil N. Biodentine: an effective pulp capping material , *Journal of Dental Science*, 3(1), 2016,15-19
- [6] G. Bogen, N.P. Chandler, Vital pulp therapy in *Ingle's Endodontics*. Ingle JI (Ed). (6th) (edn. Onterio, BC Decker Inc, 2008), 1110-30.
- [7] S. Asgary, M. Fazlyab, S. Sabbagh, M.J. Eghbal, Outcomes of different vital pulp therapy techniques on symptomatic permanent teeth: A case series, *Iranian Endodontic Journal*, 9(2) 2014, 295-300.
- [8] P. Karthikeson, S. Jayalakshmi, Pulp Capping Agents-A Review, *Indian Journal of Pharmaceutical Science. & Reserch*, 8(6), 2016, 525-27.
- [9] Q. Asma, N. Soujanya, S. Pratapkumar, Recent Advances In Pulp Capping Materials: An Overview, *Journal of Clinical & Diagnostic Reserch*, 8(1), 2014, 316-21.
- [10] A. Jalan, M. Warhadpande, D. Dakshindas, A comparison of human dental pulp response to calcium hydroxide and Biodentine as direct pulp-capping agents. *Journal of Conservative Dentistry*, 20(2), 2017; 129-33.
- [11] K. Takashi, R. Arataebihara, A. Akira, The Use Of Lasers For Direct Pulp Capping, *Journal of Oral Science*, 57(4), 2015, 277-86.
- [12] T. Dammaschke, U. Stratmann, P. Wolff, D. Sagheri, E. Schafer, Direct pulp capping with mineral trioxide aggregate: An immunohistologic comparison with calcium hydroxide in rodents, *International Journal of Endodontics*, 36(2), 2010, 814-19.
- [13] K. Laslami, S. Dhoun, M. Karami, M. Jabri, Direct Pulp Capping with Bioactive Material: Biodentine, *EC Dental Science*, 13(2), 2017, 75-83.
- [14] X. Trans, C. Gorin, C. Willing, Effect of calcium silicate based restorative cement on pulp repair. *Journal of Dental Reserch*, 91(12), 2012 1166-71.
- [15] M. Moustafa, M. Nagawa, Kh. Ali, Wael H, Histopathological and Histochemical Evaluation of Pulpal Response to Biodentine Compared to Portland Cement in Pulpotomized Dogs' Teeth, *EC Dental Science*, 17(3), 2018, 261-72.
- [16] D. Pooja, S. Yogesh, J. Aditi, M. Praveen, R. Owais, S. Shruti, Biodentine: the new bioactive and biocompatible material of choice for direct pulp capping & Pulpotomy in curiously exposed permanent teeth: case reports, *International Journal of Applied Dental Science*, 4(4), 2018, 09-12.
- [17] M. Renato, B.M. Clovis, L. Ariadne, G. Vanessa, B.B. Roberto, I. Nitero, Histologic evaluation of pulpotomies in dog using two types of mineral trioxide aggregate and regular and white

- Portland cements as wound dressing, *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology*, 98(2), 2004; 376-9.
- [18] A. Ali, A. Farahanaz, B. Majid, M. Sayed, D. Amin, Pulp Response following Direct Pulp Capping with Dentin Adhesives and Mineral Trioxide Aggregate; An Animal Study, *Iranian Endodontic Journal*, 12 (2), 2017, 226-30.
- [19] K. Jae-Hoon, H. Jun-Bae, L. Bum-Soon, C. Byeong-Hoon, Histological evaluation of direct pulp capping with DSP-derived synthetic peptide in beagle dog. *Journal of Korean Academic Conservative Dentistry*, 34 (2), 2009, 120-8.
- [20] I. Faraco, R. Holland, Response of the pulp of dogs to capping with mineral trioxide aggregate or calcium hydroxide cement. *Dental Traumatology*, (17), 2001; 163- 6.
- [21] A.L. Briso, V. Rahal, S.R. Mestreneur, E.J. Dezan, Biological response of pulps submitted to different capping materials, *Brazilian Oral Reserch*, (20), 2006; 219-25.
- [22] Hengameh B, Mohammad H, Pouyan A, Fatemeh A, Fereshteh N, Ehsan E, Arash A, Samar E, Mohammad R, Vahid M, Mehdi S and Imad A. Human Pulp Responses to Partial Pulpotomy Treatment with TheraCal as Compared with Biodentine and ProRoot MTA: A Clinical Trial. *Endodontic Journal*, (43), 2017, 1786-91.
- [23] A. Negm, E. Hassanien, A. Aboseida, M. Nagy, Biological evaluation of a new pulp capping material developed from portland cement, *Experimental and Toxicologic Pathology* 69(1), 2017; 115–22.
- [24] A. Abu-Seida, Efficacy of diclofenac sodium either alone or together with cefotaxime sodium for control of postoperative pain in dogs undergoing ovariohysterectomys, *Asian Journal of Animal and Veterinary Advances*, (7), 2012, 180 – 6.
- [25] J.D. Bancroft, M. Gamble, *Theory and Practice of Histological Techniques*. (6th), Ed, (Churchill Livingstone, Elsevier, China, 2008).
- [26] A.L. Briso, V. Rahal, S.R. Mestreneur, E. Dezan Junior, Biological response of pulps submitted to different capping materials, *Brazilian Oral Reserch*, (20) 2006; 219-25.
- [27] D. Pooja, S. Yogesh, A. Jain, M. Praveen R. Owais, S. Shruti, Biodentine: the new bioactive and biocompatible material of choice for direct pulp capping & Pulpotomy in curiously exposed permanent teeth: case reports, *International Journal of Applied Dental Sciences*, 2018, 4(4), 09-12
- [28] P.S. Karthikeson, S. Jayalakshmi, Pulp Capping Agents-A Review, *Journal of Pharmaceutical Science & Reserch*. 8(6), 2016, 525-27.
- [29] G.A. Silva, E. Gava, L.D. Lanza, C. Estrela, J.B. Alves, Subclinical failures of direct pulp capping of human teeth by using a dentin bonding system, *Journal of Endodontics*, 39(2), 2013, 182– 89.
- [30] M.L. Accorinte, A.D. Loguercio, A. Reis, E. Carneiro, R.H. Grande, S.S. Murata, R. Holland Response of human dental pulp capped with MTA and calcium hydroxide powder, *Operative Dentistry*, (33), 2008, 488–95.
- [31] M.N. Ahmed et al, Biological evaluation of a new pulp capping material developed from Portland cement, *Experimental and Toxicologic Pathology*, 69(3), 2017; 115-22.
- [32] F. Danesh, A. Vahid, J. Jahanbani, F. Mashhadidbbas, E. Arman, Effect of white mineral trioxide aggregate compared with biomimetic carbonated apatite on dentine bridge formation and inflammatory response in a dental pulp model, *International Endodontic Journal* 45 (1), 2012; 26-34.
- [33] P. Marijana, D. Vesna, P. Branislav, P. Bogomir, J. Vukoman, Z. Slavoljub, Direct pulp capping using biodentine, *Serbian Dental Journal*, 61(2), 2014, 65-74.
- [34] D. Tziafas, A.J. Smith, H. Lesot, Designing new treatment strategies in vital pulp therapy, *British Dental Journal*, (28) 2000, 77-92.
- [35] N. Alicja, L. Ryta, L. Mariusz, P. Miroslaw, G. Katarzyna, S. Ewa, W. Angieszka, B. Jadwiga, The clinical and histological evaluation of direct pulp capping on human pulp tissue using a dentin adhesive system, *Biomedical Reserch International*, 2016: 259-64.

- [36] V. Stefanova, S. Tsanova, P. Atanasova, I. Borisov, T. Chaprazov, M. Tsanova, Early results after direct pulp capping with tricalcium silicate based material and mineral trioxide aggregate and ER:YAG cavity preparation in dogs, *Trakian Journal of Oral Science*, 12(1), 2014, 331-4.
- [37] K. Yoshiba, N. Yoshiba, H. Nakamura, M. Iwaku, H. Ozawa, Immunolocalization of fibronectin during reparative dentinogenesis in human teeth after pulp capping with calcium hydroxide, *Journal of Dental Reserch*, (75), 1996, 1590-7.
- [38] A. Eskandarizadeh, M. Shahpasandzadeh, M. Torabi M, M. Parirokh, A comparative study on dental pulp response to calcium hydroxide, white and grey mineral trioxide aggregate as pulp capping agents, *Journal of Conservative Dentistry*, 14(4), 2011; 351-55.
- [39] X.V. Tran, C. Gorin, C. Willig, B. Baroukh, B. Pellat, F. Decup, Effect of a calcium-silicate-based restorative cement on pulp repair, *Journal of Dental Reserch*, 91 (2), 2012, 1166-71.
- [40] N. Takahashi, B. Nyvad, The role of bacteria in the caries process: Ecological perspectives, *Journal of Dental Reserch*, 90 (3), 2011, 294-303.
- [41] W. Peng, W. Liu, W. Zhai, L. Jiang, J. Chang, Effect of tricalcium silicate on the proliferation and odontogenic differentiation of human dental pulp cells, *International Endodontic Journal*, 37(1), 2011, 1240-6.
- [42] B. Sepideh, F. Mahta, H. Haleh, M. Faramarz, M. Pouria, Histological evaluation of single and double visit direct pulp capping with different materials on sound human premolars: A randomized controlled clinical trial, *Iranian Endodontic Journal*, 10 (2), 2015, 82-8.
- [43] M.L. Accorinte, A.D. Loguercio, E. Reis, E. Carneiro, R.H. Grande, S.S. Murata, Response of human dental pulp capped with MTA and calcium hydroxide powder. *Operative Dentistry*, (33), 2008, 488-95.
- [44] A. Nowicka, M. Lipski, M. Parafiniuk, K. Sporniak-Tutak, D. Lichota, A. Kosierkiewicz, Response of human dental pulp capped with biodentine and mineral trioxide aggregate. *International Endodontic Journal*, 39(1), 2013, 743-7.
- [45] TS da Fonseca et al. In vivo evaluation of inflammatory response and IL-6 immunoexpression promoted by Biodentine and MTA Angelus, *International Endodontic Journal*, 49(2), 2016, 145-53.
- [46] B. Tabarsi, M. Parirokh, M.J. Eghbal, A.A. Haghdoost, H. Torabzadeh, S. Asgary, A comparative study of dental pulp response to several pulpotomy agents, *International Endodontic Journal*, 43(2), 2010, 565-71.
- [47] S. Asgary, M.J. Eghbal, M. Parirokh, Sealing ability of a novel endodontic cement as a root-end filling material, *Journal of Biomedical Material Reserch A*, 2008.