International Journal of Applied Dental Sciences 2020; 6(3): 232-238

ISSN Print: 2394-7489
ISSN Online: 2394-7497
IJADS 2020; 6(3): 232-238

www.oraljournal.com
Received: 08-06-2020
Accepted: 09-07-2020

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A histological study to evaluate the dental pulp tissue reaction to platelet rich fibrin (PRF) application in comparison with mineral trioxide aggregate (MTA) in direct pulp capping

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Abstract
Aim of this study: evaluate the tissue reaction of the Mineral Trioxide Aggregate (MTA) and compare it with Platelets Rich Fibrin (PRF) as a pulp capping material for rabbits in terms of the inflammatory reaction and formation of the dentinal bridge.
Materials and methods: 5 New Zealand male-rabbits were chosen within a 3-week observation period; the work was done on the four upper and lower incisors of each rabbit. The group consists of 20 rabbit-teeth. MTA was randomly applied to 10 teeth (5 upper incisors, 5 lower incisors), PRF was also randomly assigned to the other 10 teeth (5 upper incisors, 5 lower incisors). A drill were used to prepare a V class preparation on the buccal surface of the incisors until mechanical pulpal exposure has performed, hemostasis were done to the exposure site, and the materials of the study were applied, all the teeth groups were restored with glass ionomer cement (GIC). After three weeks, the animals were stripped; incisors were extracted and embedded in 10% formalin, later histological cross sections were obtained for microscopic study.
Results: no statistically significant differences were obtained between MTA and PRF at 95% confidence level for the inflammatory reaction and formation of the dentinal bridge.
Conclusions: similarity of PRF and MTA as pulp capping materials.

Keywords: Mineral trioxide aggregate (MTA), platelet rich fibrin (PRF), direct pulp capping, immature permanent teeth, rabbit teeth

1. Introduction
Premature teeth remain the focus of attention of many researchers, the need to preserve the pulp vitality was and still is the goal of many research that shed light on conservative treatments, and these measures to preserve pulp vitality are preferred to radical root treatment or any other pulpal procedures that can be complex, costly, and the longest time consuming [1].
Since the mid-nineties, the MTA has been considered a reference material for all conservative biomedical treatments, with high success rates reaching 90% - 100% in clinical, radiological and histological studies, and despite the tremendous success achieved by the MTA, some of the negatives contributed to reducing From its application, including: long hardening time (3 hours), difficulty in application, and high cost [2].
Platelet regeneration techniques were introduced in the 1970s, and it was noted that they contain growth factors responsible for collagen increase, cell division, vascular growth and cell differentiation. One of the most recent applications of biological tissue engineering is: platelet rich plasma (PRP) and fibrin-rich platelet (PRF) [3].
Recently, these techniques were used on the pulp of young teeth, and it was noted that the pulp would heal, continuing to allow the growth of the tooth root until completed, and that platelet regeneration techniques contained cells capable of forming hard tissues [4].
Direct pulp coverage is the treatment of pulpal exposure (due to necrosis, preparation, or trauma), after clinical and radiological diagnosis to confirm that the pulp is intact and uninfected with irreversible pulpitis [5].
MTA achieved a coverage rate of 80.3% success rate, outperforming calcium water, which achieved a 68.5% success rate, and they were applied to direct endodontic coverage on teeth within a 24-month observation period [6].

1.1 MTA (Mineral Trioxide Aggregate)

The MTA is a powder made of fine, hydrophilic particles of three-calcium silicates, tri-calcium aluminum, tri-calcium oxide, silica oxide and bismuth oxide, which added to make the material radiopaque, and contain small amounts of other metallic oxides. The MTA powder is mixed with distilled water, in a 1: 3 ratio of powder to liquid. The hydration of the powder creates a colloid that gradually increases its durability and turns into a solid structure within approximately three hours [7].

1.2 Platelets Rich Fibrin (PRF)

The first use of PRF was in 2001 by Choukroun, specifically in the field of oral and maxillofacial surgery, which is a new generation of platelets [3].

In the last two decades, an understanding of the biological and physiological properties of PRF has resulted in the healing and regeneration of tissues for more successful therapeutic applications, and it has been called the second generation of platelet concentrate which has been shown to have many benefits compared to the traditional method of preparing platelet rich plasma (PRP). This technique is simple, inexpensive, easy to prepare, and there is no biochemical treatment for blood, which makes it completely autogenous (from the patient's own blood without any additions) [8].

The white cells present in PRF's play an important role in releasing growth factors, slow polymerization and high scarring ability; which creates important role in physiological properties of wound healing and the formation of a re-molding mesh during inflammation. Studies have reported that PRF has immune and antibacterial properties that may cause granulation of leukocytes; Dohan *et al*. confirmed That during the PRF preparation process the mesh of fibrin (a natural polymerization process) was very similar to the natural mesh of fibrin, and that the PRF is able to release cytokines that give it the ability to control inflammation [9].

PRF consists of a self-platelet-rich fibrin (fibrous) template that contains cytokines, platelets, white blood cells, glycoproteins such as thrombospondin, stem cells and other growth factors; the interaction among these elements is the gold standard in tissue healing and regeneration [10].

2. Aims of the study

Histological Evaluation of MTA in comparison with Platelets Rich Fibrin (PRF) as a covering material of the dental pulp of the rabbit teeth, in term of inflammatory reaction and formation of the ivory bridge.

3. Materials and Methods

3.1 Choosing rabbit's specimen

The study was performed on New Zealand rabbits with pink eyes, broad faces and long ear. This breed of rabbits is considered to be pure; it is characterized by its calm nature, their breeding does not require long experience, in addition to their adaptation to different environmental conditions.

Five adult-male rabbits were selected, whose weight ranges between 3.5 - 4.5 kg, which corresponds to the age of one year. Females were excluded due to periodic hormonal reasons and the possibility of pregnancy during the study period.

3.2 Material- group selection:

The study was done on the upper and lower incisors of the rabbits (two upper incisors - two lower incisors) for each rabbit. The period of the observation lasted for three weeks, were:

1. MTA was applied to the upper and lower incisors for all rabbits in groups.
2. The PRF was applied to the upper and lower incisor for all rabbits in groups.

Materials were randomly distributed to the upper and lower right and left incisors based on the drawing method; in which one of the two papers were written to choose blindly from, one paper written in it "MTA" and the other was written in it "PRF". The first paper pull were made for the right incisors (upper + lower), and the second paper pull were made for the left incisors (upper + lower), every pull were approved for each Rabbit in each group.

3.3 Materials properties

Some of the materials and tools used in the tooth-work:

Metal Trioxide Complex (MTA) from the Swiss PD company, the package consists of three envelopes, each envelope contain 0.5 g of this substance in the form of a powder, in addition to a package containing 3 ml of distilled water allocated for mixing the material, figure (1).

Fig 1: MTA used in the study

Materials used in preparing PRF:

1. Blood drawing instruments: gauge 19 with needle-dry tubes to collect blood.
2. The Centrifuge, "Kubota" Japanese Company.
3. Gauze, blade and holder

Materials used for general anesthesia:

- **Sedation:** Use the pharmaceutical preparation (Zila-Jikt), which is the brand name for the drug Xylazine.
- **Anesthesia:** The use of the drug (Ketamine-Saad), which is the brand name for the drug Ketamine.

3.4 Preparing the animal of the experiment

After knowing the weights of rabbits, the animal xylazine was given intramuscularly at a dose of 5 mg / kg by the specialist veterinarian. Xylazine affects the central and peripheral nervous system, as it works on pre- and post-synaptic receptors, and is used mainly for sedation, anesthesia, analgesia, and muscle relaxation, but it causes side effects such as decreased heart rate and hypotension in the event of an increased dose.

After five minutes, the rabbit is given a muscle injection of ketamine hydrochloride 10 mg /kg in the thigh muscle, this dose leads to a similar condition to sleep, accompanied by muscle relaxation resulting from activation of the conduction via the nervous synapse and causes a loss of pain sensation, but it differs from one animal to another, and this requires Use

of local anesthesia. Ketamine injections cause a decrease in heart rate, slow breathing, high blood pressure and body temperature for a short period and then followed by a moderate drop; the effect of the dose begins after giving it ten to fifteen minutes, figure(2).

Fig 2: Rabbit after obtaining complete sedation.

3.5 PRF preparation

5 ml of rabbit blood was drawn through the auric vein using a syringe carrying a gauge needle 19), then the blood was discharged into a test tube without any anticoagulants, figure (3).

Fig 3: Pulling the blood from auricular vein.

Putting the tube in the Centrifuge and graveled on the opposite side with a test tube that contains (5ml) of water to achieve the balance during the rotation of the Centrifuge. The blanching is calibrated at 3000 rpm for a period of 10 minutes.

The PRF was pulled with sterile clumped and placed within sterile gauze to remove the suspended liquids within the fibrin fibers and to keep-only the PRF film, figure (4).

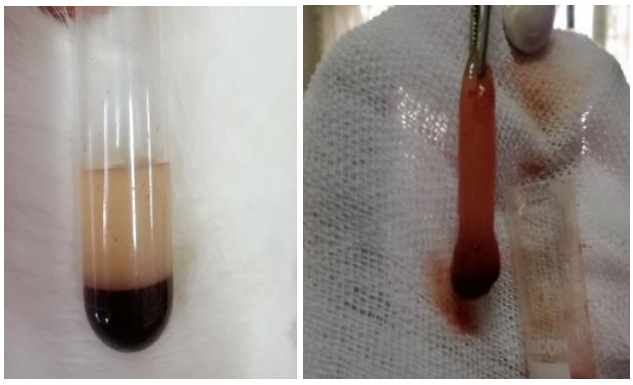


Fig 4: Obtained PRF Membrane.

3.6 Intraoral preparations

Prior to the dental preparation procedure, 5% Povidone solution was used to clean perioral area. Local anesthesia with lidocaine 2% with adrenaline 1/80000 were applied for the work area, which performed in the oral vestibule corresponding to the upper and lower teeth above the periosteal.

V class preparations were prepared with a drill on the incisors of the experiment animals. A hole were prepared using a small round diamond bur with a diameter of 1 mm installed on a high-speed hand peace with continuous cooling with water; thus we would have obtained preparation drilling of uniform sizes at all rabbit teeth included By research. Then, the round drill was replaced by a conical one with a tapering head; this drill is used for deepening of the pre-prepared hole towards the pulp horn, and the pulp exposure will occurred to the amount of drill head, figure (5). Then, thin the exposed area was covered with moisture cotton ball with 0.9% saline solution.

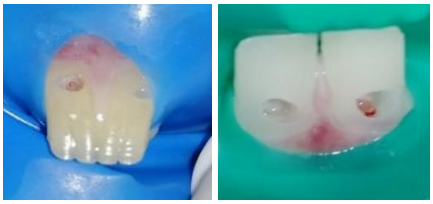
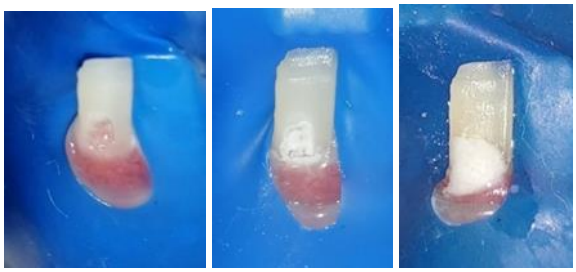


Fig 5: Exposure area made on upper and lower incisors.

After obtaining hemostasis, we applied the following*:*

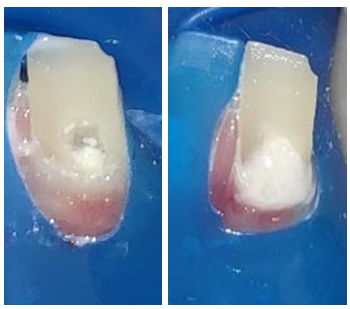
- PRF Application: After obtaining the PRF film it was dried (removal of suspended liquids within the fibrin fibers and obtaining only the pure film), then cutting the PRF to obtain an appropriate amount fitting the size of the pre-prepared exposure, a layer of the PRF was placed on the exposure area and then covered with a layer of MTA and glass ionomer cement restoration, figure (6).



(1) (2) (3)

Fig 6: (1) Applying PRF to exposure area (stage 1). (2) Applying MTA over PRF (stage2). (3) Applying GIC as a restoration (stage3).

- Application of MTA: The MTA's mixture was mixed with distilled water in a ratio of 3: 1 (according to the manufacturer's instructions, which was characterized by a hardening speed) on a glass plate with the help of metallic spatula, then the mixture was transferred by a metal holder to its place of application, and MTA were applied to the exposure, then the whole area were restored with glass ionomer cement, figure (7).



(1) (2)

Fig 7: (1) Applying MTA to exposure area (Stage 1). (2) Applying GIC as a restoration.

3.9 Histological study

Experimental animals were sacrificed after 3 days and 3 weeks by separating the head, spreading the bone pieces of the upper and lower jaws loaded with the top and bottom incisors, and placed directly in the 10% extended formalin fluid for 48 hours at room temperature, transferred to special containers containing formalin concentration (10%) with nitrogen acid with a concentration of (15%) in order to lose the mineral salts while preserving the samples of each group in a special container [11].

The evaluation of samples and the histological diagnosis were based on the following criteria

- Inflammatory response to dental pulp [12]:

Grade 0: the absence or presence of very few inflammatory cells circulating near the exposure area.

Grade 1: is a low prevalence of inflammatory cells, whether they are white single or multi-core.

Grade 2: average intensity of inflammatory cells in only a third of the coronary pulp.

Grade 3: heavy proliferation of inflammatory cells in a third or more of the coronary pulp.

Grade 4: pulp necrosis.

- Formation of the dentinal Bridge [13]:

Grade 0: no formation for hard tissues.

Grade 1: incomplete formation of hard tissue.

Grade 3: formation of a thick layer of hard tissue, figure (8,9).

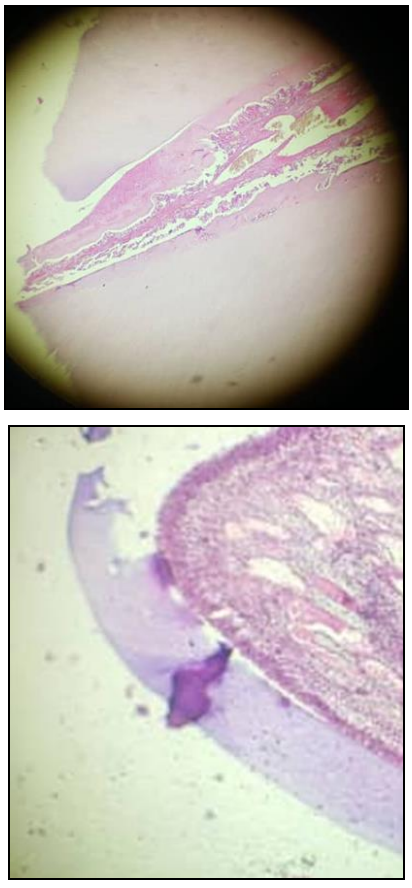


Fig 8: MTA after three weeks with formation of a complete dentinal bridge with a slight spread of inflammatory cells.

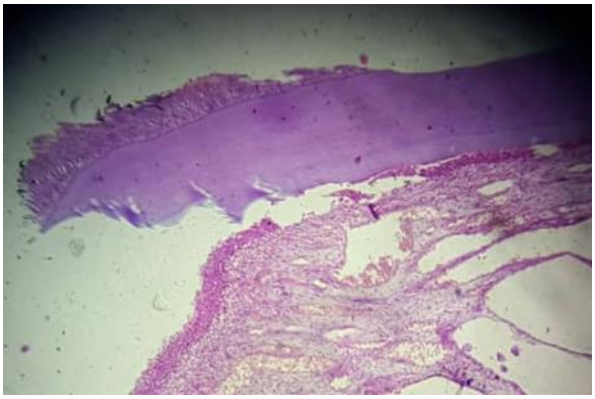


Fig 9: After three weeks, the PRF forms a complete dentinal bridge with a slight spread of inflammatory cells.

4. Results and Statically Analysis

4.1 Descriptive statistics for the inflammatory cell response scale

Table 1: Absolute and relative repeated distributions of the recorded levels of the inflammatory cell response scale in the teeth to which MTA is applied.

	Teeth	Values	3 Weeks	
			Repetition	Relative Frequency %
MTA		0	0	0
		1	4	80
	Upper	2	1	20
		3	0	0
		4	0	0
		Sum	5	100
		0	0	0
	Lower	1	5	100
		2	0	0
		3	0	0
		4	0	0
		Sum	5	100

Table 2: Absolute and relative iterative distributions of the recorded levels of the inflammatory cell response scale in the teeth to which the PRF applied.

	Teeth	Values		3 Weeks
			Repetition	Relative Frequency %
PRF		0	0	0
		1	3	60
	Upper	2	2	40
		3	0	0
		4	0	0
		Sum	5	100
		0	0	0
	Lower	1	4	80
		2	1	20
		3	0	0
		4	0	0
		Sum	5	100

4.2 Analytical statistic for the inflammatory cell response scale

When comparing the effect of the materials used in the study (MTA - PRF) on the inflammatory response scale we found:

Table 3: Results of using the Mann Whitney Test when comparing the effect of the two substances used in the study (MTA - PRF) with regard to the inflammatory response scale.

Time	Arch	Used Materials	Number	Average Rank	Total Ranks	mann whitney U value	P-Value	Indication of differences
3 weeks	Upper	MTA	5	5.00	25.00	10		no significant differences
		PRF	5	6.00	30.00		0.513	
	Lower	MTA	5	5.00	25.00	10		no significant differences
		PRF	5	6.00	30.00		0.317	
		PRF	5	5.50	27.50			

From the above table, when comparing the effect of both MTA and PRF on the inflammatory cell response scale, we did not notice statistically significant differences where the value of the significance level (P-value) was greater than the value 0.05 using the Mann-Whitney test to study The significance of the bilateral differences in the frequency of the inflammatory cell response scale. That is, at the 95% confidence level, there are no statistically significant differences in the frequency of the inflammatory cell response scale between the effect of each of the two materials applied to the teeth, namely MTA and PRF in the upper and lower jaws, in the study time of (3 Weeks).

4.3 Descriptive statistics for scale of ivory bridge formation

Table 4: Absolute and relative iterative distributions of the recorded log values for the scale of dentinal bridge formation in the teeth which the MTA were applied.

	Teeth	Values		3 Weeks
			Repetition	Relative Frequency %
MTA		0	0	0
		1	1	20
	Upper	2	4	80
		Sum	5	100
		0	0	0
	Lower	1	1	20
		2	4	80
		Sum	5	100

Table 5: Absolute and relative iterative distributions of the recorded log values for the scale of ivory bridge formation in the teeth to which PRF is applied.

	Teeth	Values		3 Weeks
			Repetition	Relative Frequency %
PRF		0	1	20
		1	1	20
	Upper	2	3	60
		Sum	5	100
		0	0	0
	Lower	1	2	40
		2	3	60
		Sum	5	100

4.4 Analytical statistics of scale of ivory bridge formation

Comparison between the effect of the materials used in the study (MTA - PRF) on the scale of dentinal bridge formation*:*

Table 6: Results of using the Mann Whitney Test when comparing the effect of the two materials used in the study (MTA-PRF) in relation to the scale of the dentinal bridge formation.

Time	Arch	Used Materials	Number	Average Rank	Total Ranks	Mann Whitney U Value	P-Value	Indication of differences
3 weeks	Upper	MTA	5	6.10	30.50	9.5	0.439	no significant differences
		PRF	5	4.90	24.50			
	Lower	MTA	5	6.00	30.00	10	0.513	no significant differences
		PRF	5	5.00	25.00			
		PRF	5	5.50	27.50			

From the table above, when comparing the effect of each of the two materials applied to the teeth, namely MTA and PRF on a scale that forms an dentinal bridge, we did not notice statistically significant differences where the value of the level of significance (P-value) was greater than the value 0.05 using the Mann- test Whitney to study the significance of bilateral differences in scale repeats of an ivory bridge. That is, at the 95% confidence level there are no statistically significant differences in scale repetitions of an dentinal bridge between the effect of each of the two materials applied to the teeth namely MTA and PRF in the upper and lower jaws in the studied time (3 weeks).

5. Discussion

5.1 Discussion of the results of platelet-rich fibrin PRF

The platelets and white blood cells present in the PRF have a

key role in triggering growth factors and anti-bacterial immune cells and immune cytokines; perhaps this is an explanation for the presence of mild inflammation of the PRF-covered pulp tissue [14].

The current study found in the 3-week observation period that the severity of the inflammatory response was low; possibly due to a gradual decrease in the release of cytokines and growth factors [14-15].

The results of the 3-week observation period differed with the study of Tabatabayi, who applied PRF as a covering material for pulp amputation on dog's teeth, where he found a light to moderate inflammatory response after three weeks [15];and this can be explained by the ability of the dental pulp on a quick recovery in rabbits.

We noticed that the formation of the dental bridge at the site of the pulp wound is a good sign of the pulp's ability to recover and repair the exposure area [16]; these results can be explained by the ability of PRF to regenerate cell activation. It causes proliferation of pulp tissue cells, increased protein transit and alkaline phosphatase activity [17]; and the growth factors in it help differentiate stem cells into the ivory photocytes responsible for formation of the dentinal Bridge [15].

The PRF liberates growth factors, the most important of which are the TGF-β1 beta-transforming growth factor and the VEGF-endothelial vascular growth factor that promotes the proliferation of Mesenchymal Stem Cells (MSCs) in the dental pulp and guides them into the formation of the pre-odontoblast cells. These growth factors gradually increase their liberation and then decrease moderately, this mean that their liberation lasts for a period ranging between at least one week and 28 days at most [18].

5.2 Discuss the results of MTA

The results of our study were in agreement with Chacko in 2006, who found that MTA caused low levels of pulpal inflammation, knowing the observation period that the researcher approved was 4 and 6 weeks [19]. While in the study of Tabarsi *et al*. it was found that MTA caused mild pulpitis when applied to dog teeth in an 8-week observation period [20].

The current study showed a complete formation of dentinal bridge in 3 weeks, and the beginning of the formation of dentinal bridge. This explains that the application of the MTA on the pulp contributes to the release of growth factors from the dentinal core such as TGF, which in turn stimulates stem cell migration and differentiation into odontoplasts that form the dentinal bridge [21].

We agreed with the findings of the Zarrabi *et al*. who found that MTA was biologically accepting and inducible in capacity to form dentinal bridge after a 2-week and 8-week observation period [22].

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