

## EFFECT OF INJECTABLE-PLATELET RICH FIBRIN ON MARGINAL ADAPTATION OF BIOACTIVE MATERIALS USED AS DIRECT PULP CAPPING; AN EXPERIMENTAL ANIMAL STUDY

Nirvana Khalaf Mansour<sup>1</sup>, Mohammed Abdullah Hashem<sup>2</sup>, Marwa Sharaan<sup>3</sup>, Dalia Mokhtar Fayyad<sup>4</sup>

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Mineral Trioxide Aggregate,  
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• E-mail address:

Dr.nirvana34@gmail.com

1. Postgraduate student in Endodontic Department, Faculty of Dentistry, Suez Canal University
2. Lecturer in Surgery, Anesthesiology and Radiology, Faculty of Veterinary, Suez Canal University
3. Associate Professor of Endodontic, Faculty of Dentistry, Suez Canal University
4. Professor of Endodontic, Faculty of Dentistry, Suez Canal University

### ABSTRACT

**Introduction:** Vital pulp therapy has been known as one of the treatment options to preserve pulp vitality after being exposed by trauma or caries. **Aim:** This experiment explored the effect of injectable-Platelet Rich Fibrin on marginal adaptation of two pulp capping agents (Mineral Trioxide Aggregate and Bioactive Bone Graft). **Materials and methods:** A total of 64 teeth were used out of 8 healthy male beagle dogs. The teeth were randomly assigned into four groups, they were exposed and capped with different capping agents. Group A; capped with Mineral Trioxide Aggregate (MTA), Group B; capped with MTA+ i-PRF, Group C; capped with Bioactive Bone Graft (BBG), Group D; capped with BBG+i-PRF. Finally the access cavity was restored with Intermediate Restorative Material (IRM). At each predetermined interval, the dogs were sacrificed (1 month, and 3 months). The samples were then prepared for electron microscopic scanning evaluation. To compare between the gap percentage of four groups at each interval, Kruskal-wallis test; was used. Mann-Whitney U test; was used to pair-wise comparison when Kruskal-wallis test is significant. Bonferroni's correction was utilized for the pair-wise comparisons. Statistical significance was considered at  $P < .05$ . **Results:** The data revealed that after one and three months the best values were recorded in groups B (MTA+ i-PRF) and D (BBG+ i-PRF), in relation to the lowest gap area between the capping materials and dentin, followed by group C (BBG), with the least value recorded in group A (MTA). **Conclusion:** the findings from the current study suggested that i-PRF provided a better marginal adaptation of either MTA or BBG to the pulp and dentin, which improved with time from one month to three months.

### INTRODUCTION

Direct pulp capping (DPC) procedure is employed when the essential asymptomatic pulp is visibly subjected to caries or trauma or to misadventure during tooth preparation or removal of caries <sup>(1)</sup>. It includes the application directly to the exposed pulp of a bioactive substance, accompanied by the immediate application of a permanent restoration. Calcium hydroxide ( $\text{Ca}(\text{OH})_2$ ) has historically been considered the gold standard <sup>(2)</sup>, it revealed some disadvantages in long-term clinical observations: like poor adherence to dentinal walls, numerous tunnel defects inside the established dentinal bridges <sup>(3)</sup>, inferior sealing ability, dissolution upon time <sup>(4)</sup> and lack of antibacterial functionality. A brand new generation of materials has therefore been seen, resulting

in more predictable clinical outcomes, namely calcium silicate materials (CSMs) <sup>(5)</sup>. Their predominant function was clarified by their high biocompatibility, intrinsic osteoconductivity and the capacity of the human body to induce regenerative reactions, namely improved quality dentin bridges and improved sealing of the pulp-capped site <sup>(6)</sup>. The foremost common example of CSMs is MTA, its biocompatibility and sealing capacity resulting from the interaction of calcium ion that is released from the fabric with phosphates found in tissue fluid, inducing the formation of hydroxyapatite crystals. A main feature liable for the chemical seal between the dentinal walls and MTA is this apatite layer formation <sup>(7)</sup>. As alternative pulp capping agents, calcium phosphate ceramics and hydroxy-apatite have been advocated. DM Bone is a ceramic made up of two-phase silicone, 60 percent hydroxyapatite and 40 percent beta-tricalcium phosphate ( $\beta$ -TCP), with a perfect 60:40 ratio of HA to  $\beta$ -TCP. Under normal physiological conditions, hydroxyapatite content is absorbed very slowly, but beta-tricalcium phosphate is typically absorbed within 6 weeks of implantation. The material shows the ability to dissolve, break down, allows new bone formation and remodeling to take place when a mix of biodegradable hydroxyapatite/beta-tricalcium phosphate ceramics is employed. With none interference, optimum mechanical strength is thus obtained, with interconnected pores of 100~600 microns in diameter, the DM bone porosity is 70 percent. Ultimate room for osteoblast migration, vascularization and bone formation <sup>(8)</sup> is given by this high porosity.

Among the newly introduced approaches to platelet-rich fibrin (PRF) tissue regeneration, multiple surgical applications such as orthopedic surgery, cardiovascular surgery, and maxillofacial surgery have a significant impact on healing <sup>(9)</sup>. Choukroun et al. first identified it in 2001 and named it the platelet concentrate of the second generation. It has

some advantages over Platelet Rich Plasma that is typically prepared <sup>(10)</sup>. There is no need for thrombin and calcium chloride for activation during the preparation of PRF, and the procedure is much simpler with less time than the preparation of PRP gel. A platelet-rich fibrin (PRF) liquid formulation called injectable-platelet rich fibrin (i-PRF) was investigated in this study without utilization of anti-coagulants <sup>(11)</sup>. Since 2010, an idea has been shown using autologous fibrin glue to provide growth factors-enriched bone graft matrix (also referred to as 'sticky bone'), it has many advantages, providing bone graft stability within the defect, thereby accelerating tissue healing and minimizing bone loss during the healing process. The resulting sticky bone is moldable in-order that the micro and macro movement of the grafted bone is prevented. In its fibrin network, it also traps platelets and leukocytes, is safe and prevents the growth of soft tissues in graft <sup>(12)</sup>. Up to our knowledge, none of the published studies investigated the marginal adaptation of adding i-PRF to either MTA or BBG, the null hypothesis of this study was that there is no significant difference between two pulp capping materials (MTA or BBG) after mixing with i-PRF in case of marginal adaptation of the experimental materials to either the pulp or dentin, after one or three months. Therefore; this study was conducted to evaluate the effect of platelet rich fibrin on marginal adaptation of different bioactive materials (MTA or BBG) used as direct pulp capping agents.

## MATERIALS AND METHODS

### 1. Study design:

This study was approved by the Ethical Committee of the Faculty of Dentistry, Suez Canal University (no.89/2018).

## Sample size determination

Eight healthy dogs with complete set of permanent dentition weighing 14-16 Kg, and aged between 10-18 months were used. Four upper and lower incisors were used, for a total of eight teeth in each dog (total sample size = 64 and 16 positive and negative control). **F tests – ANOVA (Yu et al., 2016)**, were conducted in to determine a sufficient sample size.

G\*Power version 3.1.9.2, Faul et al., (2007), University of Kiel, Germany, was used for sample size calculation. 1992-2014 Copyright

(c) <sup>(13)</sup>. Using alpha ( $\alpha$ ) level 0.05 and beta ( $\beta$ ) level 0.05, the effect size was 0.25, i.e. power = 95%; the approximate minimum sample size (n) was a total of 48 samples for four experimental groups over a single duration.

## Pulp capping procedure:

### 1. Pre-operative care:

The dogs were bathed in Diazinon (Neocidal EC, Ciba-Geigy, Switzerland) at a concentration of 1/1000 ml of water for this experiment at the Department of Surgery, Anaesthesiology, and Radiology, Faculty of Veterinary Medicine, Suez Canal University, and were then subcutaneously injected with Ivermectin (Ivomec MSD Merk & Co. Inc. U.S.A) at a dosage of 200 mg/kg body weight for control of external and internal parasites. They were served daily soft food three times a day. All the time, there was pure water available. Under the care of an expert veterinarian, all the dogs were monitored regularly for any pathological conditions.

### 2. Grouping of teeth:

The teeth were divided into four experimental groups (n=8) according to pulp capping materials and two control groups (n=2) as the following;

Group A: MTA + distilled water, group B: MTA + PRF, group C: BBG + sterile saline, group D: BBG + PRF. (negative control), teeth with no exposure and pulp capping and (positive control), teeth were capped with Teflon disc. Finally, all cavities were restored with IRM (Dentsply, Charlotte, U.S.A). Based on the observation times, each group was then subdivided into two subgroups. Group T1: 4 dogs were scarified after 1 month and group T2: 4 dogs after 3 months for evaluation. The teeth were randomly assessed by the three different observers, without knowing which material was used. The only one who knew whether A, B, C or D represented which material was the allocator.

### 3. Anaesthesia:

1.0 mL of intramuscular diazepam (Chimidarou, Tehran, Iran) was injected for sedation half an hour before the operation, accompanied by an intramuscular injection of 10 mg/kg of ketamine HCL anesthetic agent (Rotex Medica, Germany) and 1mg/kg of xylazine (Rotex Medica, Germany) <sup>(14)</sup>. Each dog was prescribed a subcutaneous injection of atropine sulphate at a dosage of 0.04 mg/kg body weight 15 minutes before the anaesthetic solution was administered. After induction of general anesthesia, 20 mL of blood was extracted from each animal and collected without any anticoagulants in two 10 mL sterile glass test tubes. Using a laboratory centrifuge, the sample was rotated at 700 rpm for 3 minutes <sup>(11)</sup>.

### 4. Cavity preparation:

With 3 percent tincture iodine, the operating area was disinfected. With the placement of gauze and cotton roll in the mucobuccal fold, the dry field was created. On the labial surfaces of all the teeth, there were prepared Class V cavities. Approximately, under abundant sterile water spray, all cavities were prepared 1mm coronal to the gingival margin with inverted cone bur at a high velocity of 30,000 rpm.

In their morphological features, the finished cavities were trapezoid with proper undercuts at the line angles to preserve the capping and temporary filling materials. Until the appearance of a pink spot, each cavity's pulpal floor was deepened. To establish pulp exposure in the center of the cavity floor, a sterile sharp probe was used. As a red mark, the exposed pulp emerged. Any bleeding was managed using cotton moistened with NaOCl until haemostasis occurred<sup>(15)</sup>.

### 5. Pulp treatment:

The exposed pulps in each quadrant were capped with one of the four tested capping materials, so that each material was represented in each dog. Group A: According to the manufacturer's instructions, MTA (Angelus, Lodrina, Paraná Brazil) was mixed with distilled water on a sterile glass slab using a metal spatula (3:1 powder-distilled water ratio) until it had the consistency of wet sand, and this mixture was placed on the sites of exposure by a fine amalgam carrier and lightly condensed with a moistened cotton pellet. Group B: Using a metal spatula, MTA was mixed with injectable-PRF on a sterile glass slab until it had the consistency of wet sand and a fine amalgam carrier placed this mixture on the exposure sites. Group C: BBG (Meta Biomed, Cheongju-si, Chungbuk, Korea) was mixed with one to two drops of sterile saline using a metal spatula to produce a putty-like paste on a sterile glass slab, which was transferred over the exposure by a fine amalgam carrier and gently condensed with a cotton pellet. Group D: BBG (Meta Biomed) was mixed with one to two drops of i-PRF using metal spatula to create a putty-like mixture on sterile glass slab, which was added over the exposure by a fine amalgam carrier and condensed gently with a cotton pellet. Negative control, teeth with no exposure and pulp capping and positive control, teeth were capped with Teflon disc. Finally, all cavities were restored with intermediate restorative material (IRM).

### Evaluation of marginal adaptation of the capping materials:

With the quick injection of 20 ml of 5 percent thiopental sodium solution through the cephalic vein, dogs were sacrificed after each observation time. Surgically, the maxilla and mandible were divided and splitted into two halves at the midline. By sectioning the jaws with a sharp saw, blocks containing a single tooth with its surrounding bone were obtained. As block pieces, the teeth and their surrounding tissues were replaced. To achieve a cross-crack of the filled cavity, each tooth was carefully notched (mark) in a bucco-lingual direction by a diamond disc. The samples were cut using a low-speed, water-cooled, 0.16 mm thick isometric diamond disk. Only the best half of the sample is used after washing the tooth halves with normal saline and the samples are then processed for scanning electron microscopic examination. Using fine abrasive paper with constant application of water, each surface was polished gently. Each specimen was placed with a thin layer of conductive tape on aluminium SEM stubs<sup>(14)</sup>. After the specimens have been mounted, they were not handled or touched again, thereby maintaining their position and orientation on the stub. The specimens were viewed and photographed at 60x, 1000x, 4000x magnification at an accelerating voltage of 20 kV, This image analysing system was calibrated to 1micrometer.

The images were transferred to a personal computer and the gap area at the filling tooth interface was calculated using Image Analysis software (Image J software, University of Wisconsin, USA). Images then were analyzed based on the presence of gaps, the ratio of gap areas to the total area of capping agent, then it was calculated and reported as a percentage. Three readings were taken for each sample then an average was calculated.

## Statistical analysis

Using the Kolmogorov-Smirnov test, all data was tested for normality by checking the distribution of data. The data was measured as mean, standard deviation (SD), range (Max-Min), etc. The data demonstrate a non-parametric distribution, so the Kruskal-wallis test was used at each time to compare the four groups. Mann-Whitney U test: When the Kruskal-wallis test is significant, it was used for pair-wise comparison. For the pair-wise comparisons, Bonferroni's correction was used for<sup>(16)</sup>.

## RESULTS

### A- Intergroup comparison

**At one month**, ANOVA test recorded that there was significant difference between groups at p-value <0.05. Analysis of Tukey's post hoc test recorded non-statistically significant difference between MTA and BBG groups and non-statistically significant difference between MTA+PRF and BBG+PRF groups. The highest values of gap area were recorded in group MTA (75.74±7.08) followed by BBG (72.57±8.11). On the other hand the lowest mean values were recorded in BBG+PRF (56.13±6.55)

group followed by MTA+PRF (61.96±5.40) group. (Table 1, Fig. 1).

**At 3 months**, the variance analysis (ANOVA test) showed that the disparity between groups was statistically significant (p=0.023). Analysis of Tukey's post hoc test recorded non-statistically significant difference between MTA+PRF and BBG+PRF groups. The highest statistically significant mean values were recorded in groups MTA (73.88±8.43) followed by group BBG (64.49±8.19). While the lowest values were recorded in MTA+PRF (51.23±5.95) and BBG+PRF group (53.31±6.58). (Table 1, Fig. 1).

### B- Intragroup comparison (Effect of time within the same group)

In MTA group, the gap area decreased by time. Lower mean value was recorded after 3 months. Paired t test revealed that there was no statistically significant difference. (p=0.65), (Table 1). In MTA+PRF, BBG, BBG+PRF groups, the gap area decreased by time. Lower mean value was recorded after 3 months. Paired t test revealed that there was a statistically significant difference. (p=0.04), (Table 1, Fig. 1)

**Table (1):** Descriptive statistics and comparison between groups for gap area (ANOVA test)

Time	Group A (MTA)		Group B (MTA+PRF)		Group C (BBG)		Group D (BBG+PRF)		P-value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
1 month	75.74 <sup>a</sup>	7.08	61.96 <sup>b</sup>	5.40	72.57 <sup>a</sup>	8.11	57.13 <sup>c</sup>	6.55	0.03*
3 months	73.88 <sup>a</sup>	8.43	51.23 <sup>c</sup>	5.95	64.49 <sup>b</sup>	8.19	53.31 <sup>c</sup>	6.58	0.023*
P-value	0.65		0.04*		0.015*		0.02*		

*C.I = confidence interval, Significance level P<0.05, \*significant*

*Tukey's post hoc test: Within, the same observation, means with different superscript letters are significantly different*

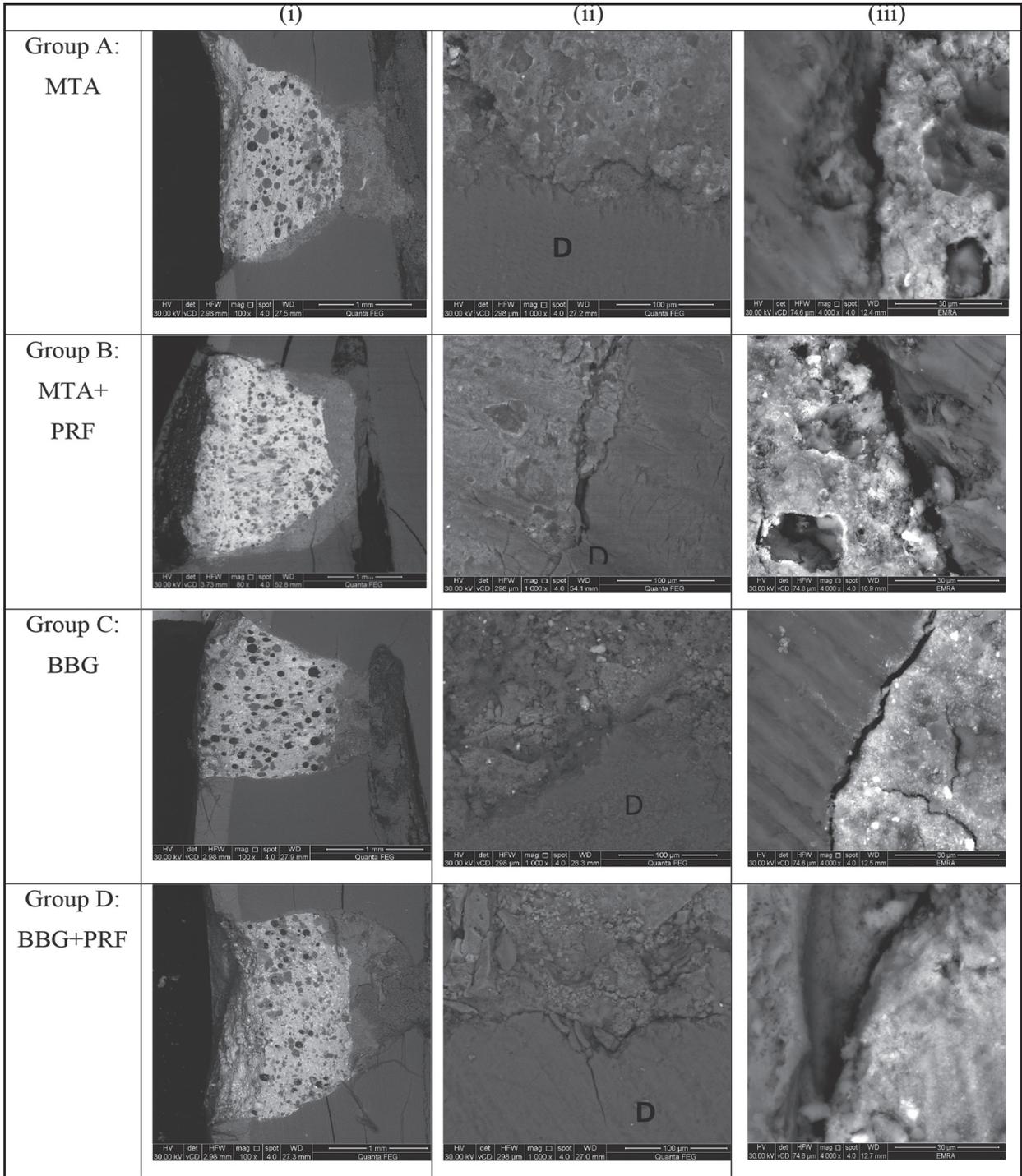


Fig.(1): SEM micrographs showing (i) cavities prepared and filled with capping materials and then IRM ( X80, X100), (ii) and (iii) represent the interfaces between pulp capping material and dentin (original magnification X1000, X4000) after one month. D: Dentin

**C-Intergroup comparison of difference by time:**

**At one month**, ANOVA test revealed that there is non-significant difference between groups at significant level  $<0.05$ . The highest values were recorded in MTA (79.25), followed by group BBG (72.60), while the lowest value was recorded in group BBG+PRF and MTA+PRF groups with values 66.85 and 68.15 respectively.

**At 3 months**, the variance analysis (ANOVA test) revealed that the difference between groups was statistically significant at  $p$  value  $<0.05$  (0.0217). Analysis of Turkey's post hoc test revealed non-significant difference between MTA+PRF and BBG+PRF groups. The highest mean values were recorded in groups MTA (77.65%) followed by group BBG (70.94%) while the lowest gap percentage was recorded in BBG+PRF group (62.25%). (Fig. 2).

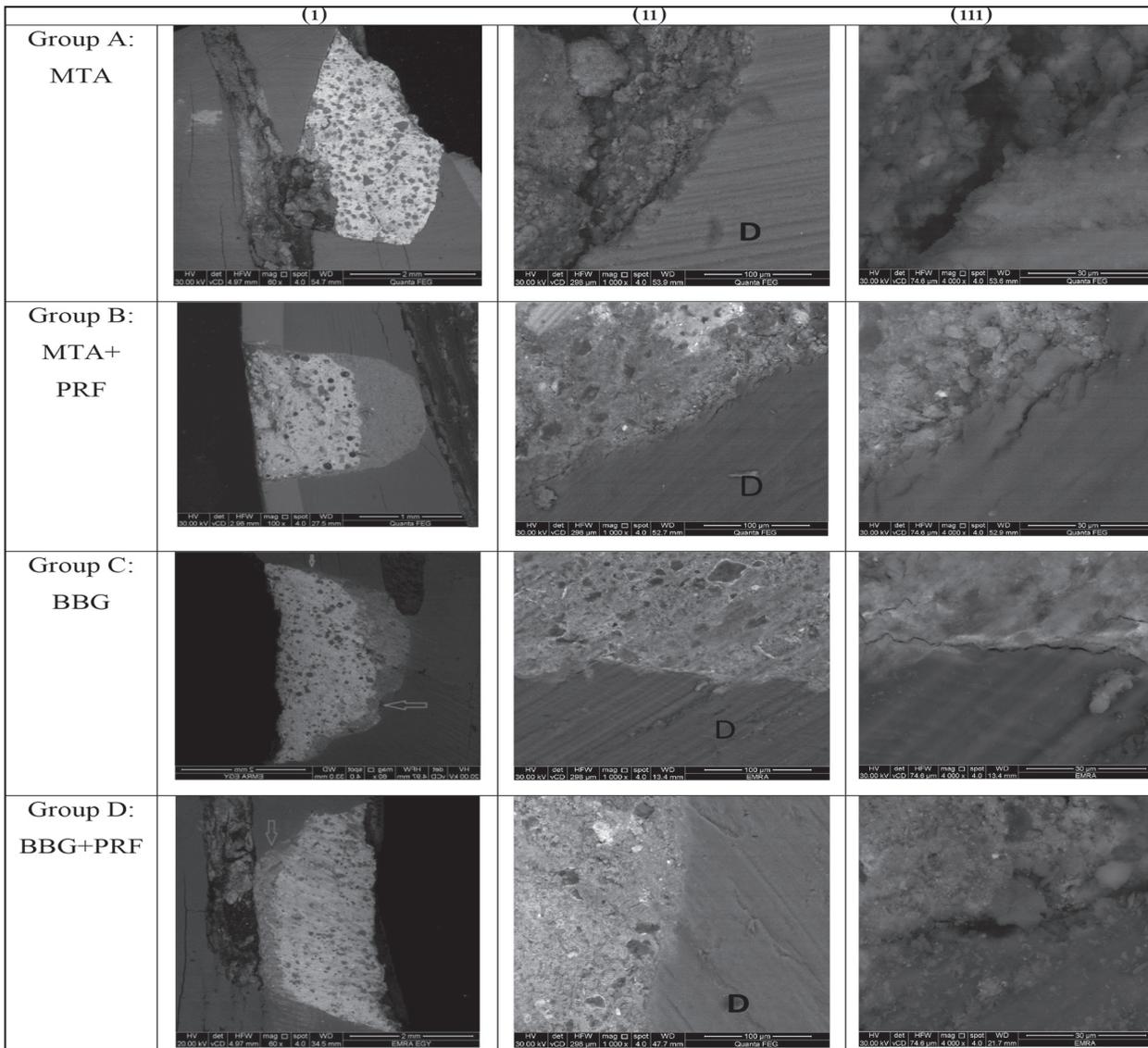


Fig. (2): SEM micrographs showing (i) cavities prepared and filled with capping materials and then IRM (X60, X100), (ii) and (iii) represent the interfaces between pulp capping material and dentin (original magnification X1000, X4000) after three months. D: Dentin.

## DISCUSSION

The effectiveness of direct pulp capping depends on numerous factors; the type of biomaterial chosen is of minor consequence and the efficiency of the material seal tested in the prevention of microbial entry is the most important consider in deciding the success of the procedure, which implies that pulpal healing occurred predictably if a bacteria-tight seal is given. Poor adhesion and/or adaptation of dentin pulp capping materials can lead to gaps and fluid variations in dentin tubules<sup>(17, 18)</sup>.

Marginal adaptation implies a material's ability to avoid the microleakage over its entire thickness. The primary explanation of most endodontic failures is the leaking of irritant products into the periradicular tissues from the infected root canal; thus, an effective seal is critical for endodontic performance<sup>(19)</sup>. Additionally, to assess the sealing capacity of the tested materials, scanning electron microscopic testing is used. Due to its high magnification and good resolution<sup>(20)</sup>.

Dogs were the experimental animals chosen to hold out this research. Since it is more beneficial to use animals in in-vivo experiments than in human beings. As it is possible to rigidly regulate the experimental conditions. In addition, in humans and a variety of laboratory animals, the mechanisms of dentin induction and synthesis are identical, although the rate of dentinogenesis may vary<sup>(21)</sup>. Also, for histopathological examination, the pulp size offers an acceptable sample. Besides, a good number of teeth in each dog is used; this allows for comparing more than one substance or technique in the same dog.

In the current study, MTA group showed the highest value of gap percent in relation to the total area, followed by BBG then groups D (BBG+PRF) and B (MTA+PRF), concerning group A (MTA), owing to

the existence of an imperfect fibrodentin bridge and the predentin layer of coarse collagen bundles that have not been calcified in certain places, this may occur<sup>(22)</sup>. It could even be due to some MTA disadvantages, such as: handling difficulties, slow setting reaction that could lead to leakage, disintegration of the surface that results in loss of marginal adaptation and consistency of the material, as setting time is one in all the foremost clinically deciding factors<sup>(23)</sup>. In addition, MTA pH changes from 10.2 to pH 12.5 in 3 h, result in high alkalinity during setting, also, due to the interaction between MTA and the dentin organic phase, resulting in the deterioration of collagen type 1, and changing the micro-hardness of dentin<sup>(25)</sup>. These findings are completely in keeping with other studies<sup>(26-30)</sup>. Conversely, in some experiments, after MTA capping, a homogeneous zone of crystalline structures was initially found along the pulp-MTA interface<sup>(31-36)</sup>, limited samples number in other studies, may be the cause for this difference. The marginal adaptation of MTA using SEM was studied by Soundappan et al.,<sup>(37)</sup>. Thirty permanent central incisors and reported that, in general comparison, when used as retrograde filling material, MTA was significantly superior in terms of marginal adaptation, but different techniques were used with different comparative materials (IRM and Biodentin). In fact, with regard to pulpal microleakage<sup>(38)</sup>, the utilization of the MTA materials as a pulp-capping agent would be more effective than Ca(OH)<sub>2</sub> materials, which could result in high solubility of Ca(OH)<sub>2</sub> subjected to dissolution over time.

However, BBG has a neutral pH during setting and is highly adaptable. It is also dimensionally stable, easy to handle and has the property of osteo-transductivity (i.e., active resorption at bony sites, facilitating bone remodelling<sup>(39)</sup>, which may explain the less gaps percentage than MTA. Also, endogenous BMPs or other proteins are hypothesized to be adsorbed and may accumulate on the

ceramic surface or into the pores of B-TCP, result in attractiveness of osteogenic cells. In turn, surface topography and ceramic inorganic ion release may also be a direct cause of the osteogenic differentiation and bone formation process.<sup>(8)</sup> These results in agreement with several studies<sup>(40,41)</sup>. However, Kiba et al.,<sup>(42)</sup> showed complete dentin bridge formation at increased rate for Poly-calcium-phosphate in comparison with HA after 4 weeks which in reverse to our results, this difference may be due to different model of study which was Wistar rat.

In regards to groups D (BBG+PRF) and B (MTA+PRF), liquid form of PRF can be used in association with bone grafts and MTA. These offer various benefits, including the encouragement of wound closure, bone growth and maturation, graft stabilization and enhancement of graft material handling properties<sup>(43)</sup>. Clinical trials indicate that the combination of bone graft and PRF growth factors could be able to improve bone density<sup>(44)</sup>. Because of the collective PRF-bone graft osteo-inductive properties, the combination of PRF and bone graft or MTA possibly facilitates improved space for cellular events needed for mineralized tissue formation. PRF serves as an osteo-inductive material because osteo-induction is a mechanism by which new bone is formed in a region where previously there was no bone<sup>(45)</sup>.

But with time there was a significant decrease in gap percentage in all groups, this improvement in the sealing ability of MTA and MTA with PRF upon time is due to its hydrophilic properties and the formation of a dentin-to-MTA interfacial layer. This interfacial layer reduces the probability of marginal percolation and increases clinical success over the long term<sup>(46)</sup>. Further hydration of MTA particles by moisture also improves compressive strength and reduces leakage<sup>(47,48)</sup> additionally MTA, in the presence of moisture, has the property of precipitating

hydroxyapatite crystals and reducing leakage. The small particle size of the MTA also increases the surface available for hydration and causes greater early strength<sup>(49,50)</sup>. This indicates the maturation and growth of apatite crystals could affect sealing efficiency and marginal adaptation of used pulp capping materials<sup>(51)</sup>. In regards to group BBG and BBG+PRF, after implantation, HA resorbs gradually and undergoes little transformation to a bone-like substance, but has greater mechanical strength compared to other calcium phosphates<sup>(52)</sup>. The high porosity usage of BCP provides optimum room for vascularization, osteoblast recruitment and bone deposition<sup>(8)</sup>.

Within the limitation of the present study, i-PRF with either MTA or BBG added the more marginal adaptation of MTA and BBG, with time the marginal adaptation was improved, so the null hypothesis was rejected.

## CONCLUSION

Injectable-PRF enhanced the marginal adaptation of MTA and BBG as direct pulp capping agents. Additionally, marginal adaptation was emphasized with time.

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