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ORIGINAL RESEARCH

Histological Evaluation of the Effect of Platelet-rich Plasma on Pulp Regeneration in Nonvital Open Apex Teeth: An Animal Study

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ABSTRACT

Aim: Platelet-rich plasma (PRP), which is a concentration of growth factors found in platelets, may be a suitable material for pulp regeneration. The aim of this animal study was a histological evaluation of PRP on pulp regeneration in nonvital teeth with immature apices.

Materials and methods: A total of 40 premolar dogs' teeth were chosen for this study. After general anesthesia, the teeth were exposed, and subsequently, pulps were removed and the cavities were opened to the oral cavity. After 2 weeks, root canals were irrigated and disinfected with sodium hypochlorite with noninstrumentation technique, and triple antibiotic paste was placed inside the canals. Cavities were sealed with a temporary restoration. About 4 weeks later, canals were irrigated again and the teeth were randomly divided into three groups. Bleeding was evoked with overinstrumentation, then experimental materials for each group [PRP, mineral trioxide aggregate (MTA), and parafilm respectively] were placed over the bleeding, and orifices were sealed with MTA and glass ionomer. After 3 months, dogs were sacrificed and the teeth were separated from the jaws and sections prepared for histological evaluation.

Results: Regeneration was shown in 44.7% of the samples. About 47.3% of the samples in the MTA group and 42.1% of the samples in the PRP group showed regeneration; however, no regeneration was observed in the parafilm group. Chi-square test showed no significant difference between groups I and II. The soft regenerative tissue included pulp-like tissue and vessels. Mineralized regenerative tissue included cementum-like, periodontal ligament-like, and bone-like tissues. No normal pulp and nerve tissue were observed.

Conclusion: Both PRP and MTA may be ideal scaffolds to accelerate the regeneration process.

Clinical significance: Pulp repair in immature permanent teeth with weak roots has a better outcome than replacement of the pulp with gutta-percha or biomaterials.

Keywords: Immature teeth, Mineral trioxide aggregate, Platelet-rich plasma, Pulp regeneration.

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INTRODUCTION

Pulp necrosis before root development is completed can stop dentin formation and root growth.[1,2] Conventionally, the treatment of immature necrotic permanent teeth is accomplished using apexification procedure (long-term usage of calcium hydroxide or one-visit artificial plug).[3] However, these procedures do not offer ideal results because the thin walls of the root canal may remain with no development and maturation, and so those teeth are prone to fracture.[1,4] Regeneration of the pulp is an alternative treatment method for these cases that could result in restoration of pulp vitality, increased thickening of the canal walls, continued root development, and elongation of the underdeveloped root.[4,5]

Hargreaves and Cohen[6] have proposed three factors contributing to this procedure success. They include

stem cells capable of formation of hard tissue, signaling molecules for cellular proliferation and differentiation, and finally, a three-dimensional physical scaffold for supporting the differentiation and cell growth. An ideal scaffold may selectively bind cells, contains growth factors (GFs), and undergoes biological degradation over time.[6,7] Platelet-rich plasma has been suggested as an ideal scaffold for regenerative treatment,[7] which contains GFs and attracts stem cells in the apical tissue.[1] Recently, Jadhav et al[8] used PRP as a matrix in revascularization of nonvital teeth and reported desired biological outcomes.

The precise nature of the tissues present in the canal and also the nature of hard tissues formed inside the canal and tissues responsible for continued root development following revascularization are unknown because few histological studies are available.[4] The aim of this study was a histological evaluation of the tissues formed in the canals after receiving regeneration treatment using PRP in nonvital teeth with immature apices.

MATERIALS AND METHODS

Three 6-month-old mongrel healthy dogs were included in the study. The study was carried out at the Animal Laboratory of the Mashhad Dental Research Center, Mashhad, Iran. The Research Council of Mashhad University of Medical Sciences (MUMS) approved the experiment (Registration number: 89825). Periapical radiographs were obtained to determine the degree of root development (Fig. 1). A total of 40 immature premolars were included in the study. Teeth were randomly divided into three experimental groups, and four teeth were assigned to control.

Before starting the study, the animals were premedicated by injection of 1 mL/10 kg body weight (150 mg/mL amoxicillin and 40 mg/mL gentamicin sulfate) (Gentamox, Gerona, Spain). The animals were subsequently anesthetized with intravenous injection of xylazine hydrochloride (0.5 mg/kg body weight) (Alfasan, Woerden, Holland) and 10% ketamine (10 mg/kg body weight) (Alfasan, Woerden, Holland). They also received 5% flunixin meglumine (ErfanDaru, Tehran, Iran) through intravenous injection to affect analgesia.

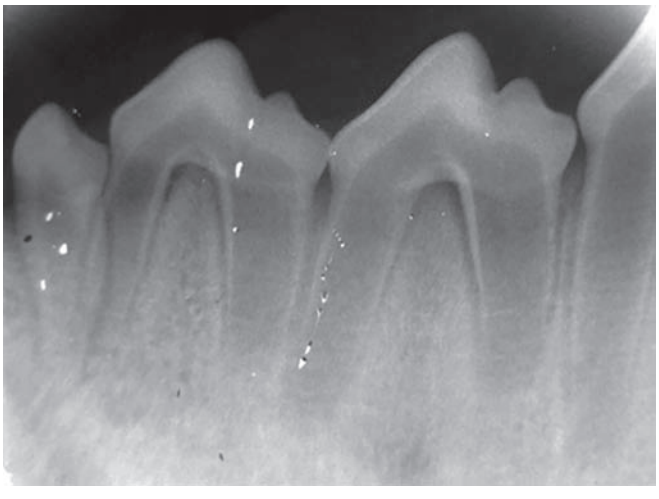

Fig. 1: Sample of initial radiographs indicating the degree of immature apex

First Session

On rinsing the dogs' mouths with 0.2% chlorhexidine mouthwash (Shahrdaru, Tehran, Iran) and performing rubber dam isolation with split dam technique, access opening was achieved by utilizing a #2 diamond bur (D + Z, Kalletal, Germany) under water spray; all overhanging dentin was eliminated from the pulp chamber roof. After determining the working length using periapical radiographs, barbed broaches (MEDIN, NovéMěstona Mora, Czech Republic) were employed to remove the radicular pulp. The aim of the first visit was inducing infection in the root canals, so in this session, access cavity was kept open to the oral environment for 2 weeks. At the end of this session, ibuprofen was administered to reduce the posttreatment pain of the animals.

Second Session

The second visit was set 2 weeks later. The purpose of this session was canal disinfection with noninstrumentation technique. After general anesthesia, outer surfaces of the teeth and oral cavity were disinfected with 0.2% chlorhexidine and working area isolated by rubber dam with split dam technique. Access cavity was irrigated and radiographs were taken for reconfirming the working length. Canals were disinfected by 20 mL of 5.25% sodium hypochlorite (NaOCl), and after final rinse by normal saline, canals were dried with sterile paper points (Aria Dent, Tehran, Iran) and triple antibiotic paste (including metronidazole, ciprofloxacin, and minocycline) prepared by the protocol of Banchs and Trope.[9] The paste was placed in canals by a carrier and was packed with large paper points. Then, the access cavities were restored using Cavit temporary filling (AriaDent, Tehran, Iran) for 4 weeks.

Third Session

At the third session (4 weeks later), the access cavity was opened after isolation with rubber dam under general anesthesia. Triple antibiotic paste was removed from the canals by irrigation with 10 mL of 5.25% NaOCl and 10 mL of 0.9 normal saline, and bleeding was evoked by 2 mm overinstrumentation beyond the apex with a #30 K-File (Maillefer, Dentsply, Ballaigues, Switzerland). Bleeding was controlled below the cementoenamel junction (CEJ), and the blood clot formed in about 15 minutes. The

samples were then randomly divided into two experimental groups and a control group:

- *Group I*: Revascularization with PRP (19 teeth)
- *Group II*: Revascularization with MTA (19 teeth)
- *Control group*: Revascularization with parafilm (2 teeth).

For PRP preparation, this protocol was used:

- Approximately 10 mL blood was drawn from the internal jugular vein of each dog into a centrifuge tube containing ethylenediaminetetraacetic acid (EDTA) to prevent blood coagulation. Collected blood was centrifuged at 2400 rpm for 10 minutes to separate erythrocytes from the platelet-poor plasma (PPP). A second centrifugation was performed on PPP at 3600 rpm for 15 minutes to obtain PRP. The release of platelet products into the supernature was induced within 10 minutes after the addition of thrombin and 10% calcium chloride to the PRP. Then, PRP was injected into the canal space up to the CEJ and allowed to clot (based on Bakhtiar et al[10] method).
- After placing the MTA (ProRoot, Dentsply, USA), the access cavity was sealed with glass ionomer cement (GC, Tokyo, Japan) (Fig. 2). After 3 months, dogs were sacrificed by vital perfusion method,[11] and samples were prepared for fixation and decalcification.
- Periapical radiographs were taken before and after the intervention and after 3 months to monitor root development. The 3-month follow-up radiographs evaluated presence or absence of apical radiolucency, apical closure, and root canal wall thickening.

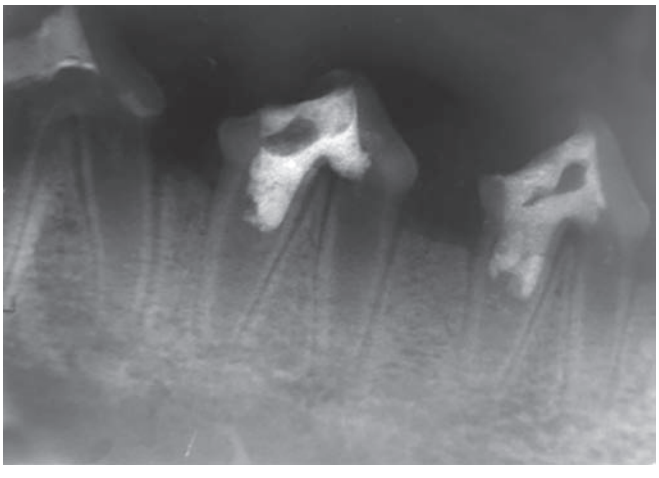

Fig. 2: Sample of final radiographs

Fixation and Decalcification

Block sections containing maxilla and mandible were placed into 10% formalin solution for 10 days. Subsequently, the sections were decalcified in 14% EDTA in agitator for 4 months. The EDTA was replaced two times each week. After decalcification was completed, the samples were transferred to the laboratory for histological sectioning. The roots were sectioned longitudinally. The samples were washed under running tap water for 5 hours, dehydrated with ascending grades of alcohol, step serial sectioning of 4 to 5 µm was performed, and the sections were stained with hematoxylin and eosin (H&E). Prepared slides were examined using a light microscope (Nikon, Tokyo, Japan) by a blinded pathologist. The presence or absence of hard tissue deposition on the radicular walls, the nature of proliferated tissue, and inflammation according to the quantity of inflammatory cells in the studied tissues were evaluated.

RESULTS

Regenerated vital tissues were shown in 44.7% of the samples. About 47.3% of the samples in the MTA group and 42.1% of the samples in the PRP group showed regeneration; however, no regeneration was observed in parafilm group. Chi-square test showed no significant difference between groups I and II ($p > 0.05$) (Table 1). The histologic evaluation revealed that the soft regenerative tissue included soft connective tissue and vessels (Fig. 3). Mineralized regenerative tissue included cementum-like, Periodontal ligament (PDL)-like, and bone-like tissues (Figs 4 and 5). No normal pulp, nerve, and dentin-like tissues were observed.

Regarding inflammation severity, the most common inflammation grade was moderate in MTA and PRP groups (71% of teeth in MTA group and 50% of teeth in PRP group) (Table 2). About 14% of teeth in the MTA group and 17% of teeth in the PRP group showed severe inflammation.

Table 1: The results of chi-square test

	Value	*df*	*Asymptotic significance (2-sided)*	*Exact significance (2-sided)*	*Exact significance (1-sided)*
Pearson Chi-square	0.106[a]	1	0.744		
Continuity correction[b]	0.000	1	1.000		
Likelihood ratio	0.106	1	0.744		
Fisher's exact test				1.000	0.500
Linear-by-linear association	0.104	1	0.748		
Number of valid cases	38				

[a]0 cells (0.0%) have expected count <5. The minimum expected count is 8.50; [b]Computed only for a 2 × 2 table; Df: Degree of freedom

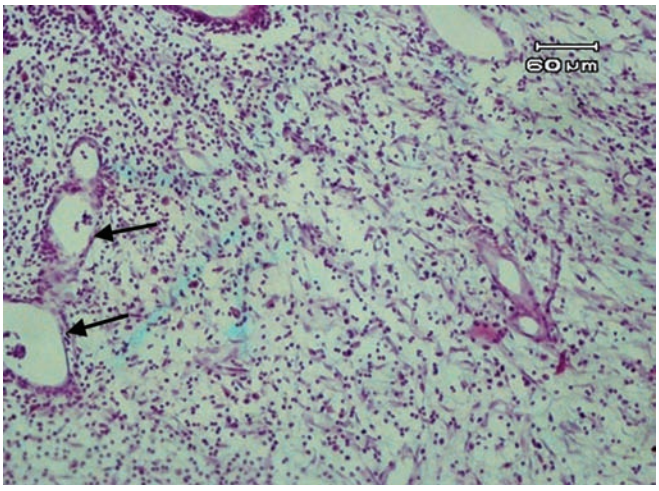

Fig. 3: Connective tissue and vessels (*arrow*) and inflammatory cells (H&E staining; magnification, 40×)

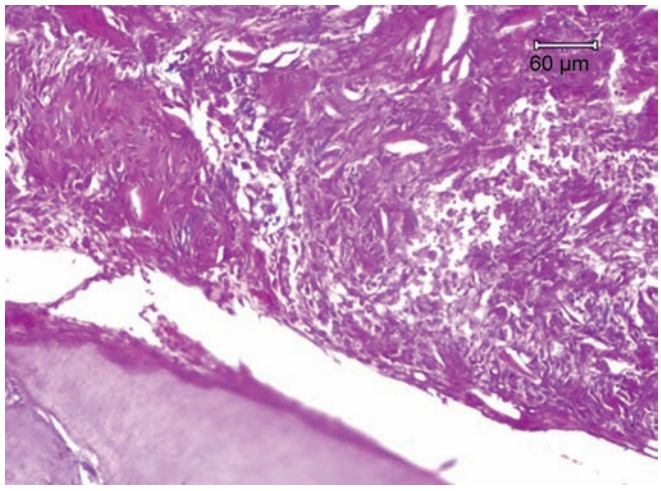

Fig. 4: Cementum-like mineralized tissue (H&E staining; magnification, 10×)

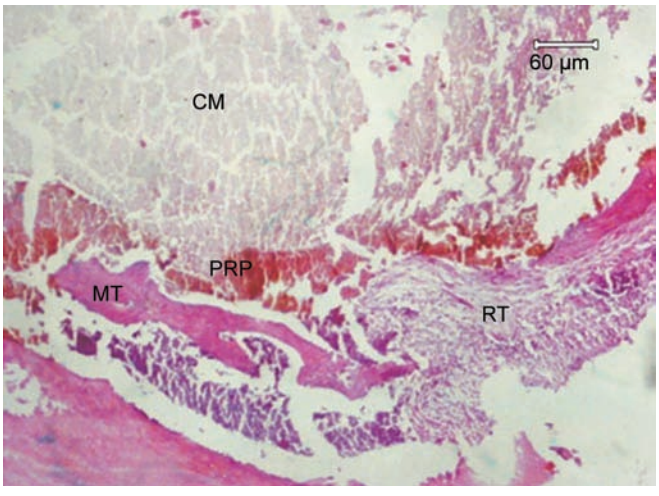

Fig. 5: Proximity of PRP and intracanal regenerative mineralized tissue and regenerative soft tissue (H&E staining; magnification, 10×)

Table 2: Severity of inflammation in the three groups

MTA (%)	15	71	14
PRP (%)	33	50	17
Parafilm (%)	0	0	0

DISCUSSION

The blood clot with the role of a suitable scaffold and also a source of mandatory stem cells has been used in some studies with successful results.[12] The absence of a blood clot has been implicated in unsuccessful cases of regenerative endodontics in some studies.[4,7,13] Ding et al[7] discussed the value of PRP usage in patients in whom it is difficult to evoke bleeding in their canals by large files. Therapeutic PRP exceeds 1 million/µL platelet (five times more than that of the normal platelet count).[14] Different GFs release through degranulation of alpha granules.[15] However, the histologic results of the present study showed no difference in revascularization with or without PRP. This may be due to this fact that platelet degranulation and release and degradation of GFs are faster in PRP and the activity of GFs may end in as early as 7 to 10 days.[16] Some authors suggest that for optimizing the effects of PRP, a sustained release form of PRP is better for use.[16,17]

No regeneration in parafilm group despite inducing bleeding suggests that there may be other important factors in the regeneration process. We used MTA as a coronal seal. This material produces necessary signal for stem cells in addition to the sealing ability.[18] It can improve regeneration by releasing GFs, such as cementum-derived growth factor, fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and bone morphogenetic proteins (BMP) from cementum matrix[19,20] and transforming growth factor beta, insulin-like growth factor, FGF, PDGF, and BMP from bone matrix.[21] Recently, it has been demonstrated that MTA can express BMP_2 and induce calcification in PDL.[22] In parafilm group, direct contact between blood cells and MTA inhibited by parafilm and releasing of this GF by MTA are impossible. Another reason for the unsuccessful regeneration in the parafilm group may be related to the questionable MTA setting adjacent to the parafilm because of no humidity.

The precise nature of the regenerated hard and soft tissues formed in the canal and responsible for continued root development following revascularization is unknown because a few histological studies are available.[4] In our study, soft regenerated tissue in canal space was soft connective tissues and vessels, and hard tissue regenerated was cementum-like, PDL-like, and bone-like tissues. This result was the same as other studies that showed that normal pulp tissue, nerve, and odontoblast cells could not be seen. The intracanal tissues have been demonstrated as cementum- or bone-like tissues, and PDL-like

fibrous connective tissue and the thickening of root canal walls do not seem to be attributed to deposition of new dentin. No pulp-like tissue with odontoblastic layer was present regardless of the intracanal medication used.[13,23-28] Vojinović and Vojinović,[29] in an animal study on dogs, showed that PDL cells have the potential of migration into the apical area of the canal after pulpectomy. By appropriate signals, PDL stem cells can differentiate into osteoblast-like and cement oblast-like cells.[30,31] It was also speculated that there might be direct in-growth of cementum and bone from the periapical tissues into the canal.[32] Skoglund and Tronstad,[33] in an animal research on pulpal changes in replanted immature teeth, showed that osteoid tissue formed inside the canal was continuous with alveolar socket bone through open apical foramen.

At this time, in animal studies, there is no histologic document showing that true pulp regeneration can be seen inside immature canals in teeth with apical periodontitis after revascularization therapy.[13,34,35] More investigations and studies are necessary to allow for maximum regeneration rates. Longer follow-up intervals also are needed to assess the possibility of continued hard tissue deposition on the dentinal walls to completely close the root canal space and for long-term prognosis of this treatment method.

CONCLUSION

According to the results of this study, it seems that in revascularization with or without PRP, repair of pulp occurred rather than its regeneration. No significant difference between PRP and MTA was seen. However, pulp repair in immature permanent teeth with weak roots has better outcome than replacement of the pulp with gutta-percha or biomaterials.

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