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ORIGINAL ARTICLE

Effects of mineral trioxide aggregate and platelet-rich fibrin on histological results of direct pulp capping in dogs

ABSTRACT

Aim: *This study aimed to compare the effects of mineral trioxide aggregate (MTA) and platelet-rich fibrin (PRF) on histological results of direct pulp capping (DPC) in dogs.*
Methodology: *In this animal study, 36 class V cavities were prepared in the incisors of adult healthy mixed-breed dogs. The teeth were then randomly divided into three groups. No material was placed in the control teeth. MTA and PRF were placed on the exposure site in groups 2 and 3, respectively. After two months, the teeth were extracted under general anesthesia and were histological analyzed regarding inflammation, calcified bridge formation and necrosis. Data were analyzed using non-parametric Kruskal-Wallis test. Pairwise comparisons were made using the Mann-Whitney U test.*
Results: *There are no statistically significant differences in terms of pulp inflammation, dentinal bridge formation and necrosis among the treatment groups capping with MTA and PRF. (P Value>0.05), however, PRF and MTA were the same in all parameters, these groups were both significantly superior to the control group.*
Conclusions: *Within the limitations of this study, PRF can be used for DPC as an alternative to MTA.*

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Introduction

The viability of dentin-pulp complex is imperative for tooth vitality and is therefore a priority in clinical control strategies. Vital pulp therapy is performed aiming to preserve the vitality of the residual healthy pulp tissue for structural and functional regeneration of dentin-pulp complex (1). Vital pulp therapy is commonly used for carious teeth or those with traumatic injuries (2). Vital permanent teeth with no sign of irreversible pulpitis or apical periodontitis may be good candidates for vital pulp therapy (3) since they have the potential for regeneration and healing of the remaining radicular tissue, and the materials used for this purpose, have to be biocompatible (4).

Vital pulp therapy requires biomaterials to cover the exposed pulp tissue as a protective barrier in direct pulp capping or pulpotomy (2, 4).

Novel biological treatments are required to resolve the pulpal inflammation and induce dentinogenesis by the pulp tissue in order to increase the success rate of vital pulp therapy.

The pulp capping agents should have bio-interactivity (release of biological ions) and also be able to produce apatite crystals (2, 5). The pulp capping agents should provide a suitable environment for regeneration of dentin-pulp complex. Moreover, they have to be non-toxic and possess antibacterial activity to induce the differentiation of odontoblast-like cells (2, 6).

Calcium hydroxide and its derivatives have been the gold standard for preservation of pulp vitality in pulp capping treatments since the 1920. However, high solubility and early loss are among the drawbacks of calcium hydroxide (1). A previous study evaluated different formulations of calcium hydroxide and indicated the formation of dentinal bridge in 50% to 87% of the cases (7). Also, teeth pulp capped with calcium hydroxide have shown its limited efficacy for pulp tissue healing and regeneration. Thus, research is ongoing to find biocompatible materials inducing pulp tissue regeneration in clinical studies.

Despite the numerous applications of calcium hydroxide, it has shortcomings such as creation of tunnel-like defects in the induced dentinal bridge, poor adhesion to dentin and absence of permanent seal.

Calcium-silicate based cements, the most important of which being mineral trioxide aggregate (MTA), are promising alternatives to calcium hydroxide for this purpose since they have shown favorable properties in animal models (8).

Following the hydration of MTA in presence of blood and other biological fluids, calcium hydroxide is formed. Moreover, MTA biologically induces the pulp cells (2, 8). MTA provides a long-term seal and is biocompatible. Freshly mixed MTA is relatively cytotoxic due to its high pH; however, it has applications in vital pulp therapy, and increased durability of teeth pulp capped with MTA has been reported (9).

Recently, platelet concentrates are increasingly used to enhance wound healing and cause soft and hard tissue regeneration after different surgical procedures. Blood clots after surgical procedures initiate the process of repair and regeneration of the hard and soft tissues. Use of platelet concentrate is one strategy to enhance natural wound healing mechanisms. A natural blood clot mainly includes red blood cells, around 5% platelets and less than 1% white blood cells. In fact, platelets not only participate in forming clot but also release important growth factors that initiate and support wound healing (10). Assessment of the mechanisms of actions of growth factors and their extraction from platelets led to the increasing use of platelet-rich plasma (PRP) in different fields of oral surgery.

Platelet-rich fibrin (PRF) is obtained by eliminating the middle layer of a centrifuged blood sample. PRF was first described by Choukran et al. (2006) and is known as the second-generation platelet concentrate. Also, PRF has numerous advantages over PRP including easy preparation and absence of blood manipulation, which indicates the obligation for it to be autologous (11).

PRF clot forms a strong natural fibrin

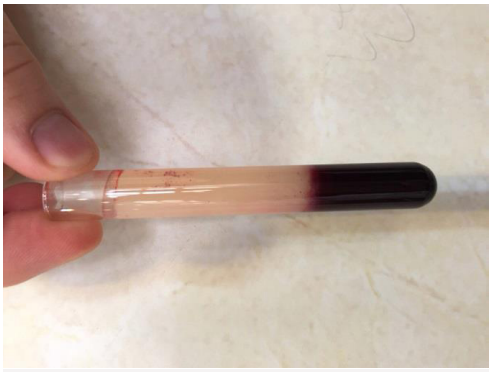


Figure 1
White buffy coat above the clot (PRF).

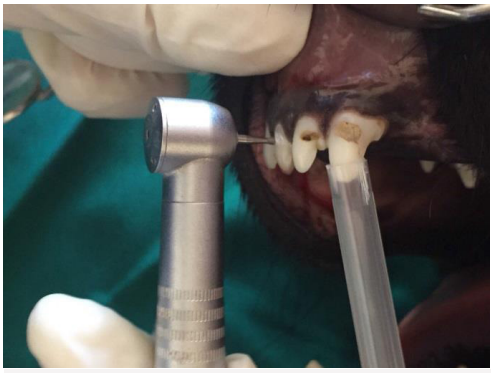


Figure 2
Cavity preparation using an inverted bur and pulpal exposure.

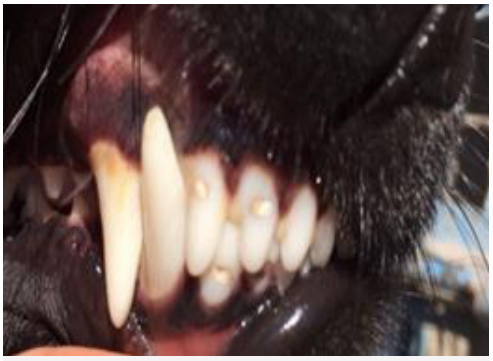


Figure 3
Restoring the cavities with Zonalin.

matrix, which includes all the platelets and growth factors of the blood sample and has a complex structure, which can best serve as a matrix for regeneration and healing. It possesses some favorable mechanical properties that other platelet concentrates do not have. PRF can serve as a biomaterial with a natural fibrin matrix to enhance micro-vascularization and guide cell migration towards the wound site. Thus, PRF has been recommended as a pulp capping agent for formation of reparative dentin or as a biomaterial for pulp regeneration (12).
This study aimed to assess the effects of application of MTA and PRF on the success rate of DPC in incisor teeth of dogs.

Materials and Methods

This animal study was conducted in line with the guidelines for the care and use of laboratory animals. In order to prepare PRF, 10 mL of blood was collected from the jugular vein of dogs. Healthy, mixed-breed adult dogs weighing 18±3 kg were chosen for this study. The dogs were refrained from eating for 8 hours prior to the surgical procedure. The blood was collected in tubes without anticoagulant agents such as EDTA. After 1 minute, the tube containing the blood was centrifuged for 10,000 cycles for 12 minutes. The white buffy coat (Figure 1) above the clot, which was PRF, was collected. General anesthesia was then induced using 0.01 mg/kg acepromazine as premedication. A combination of ketamine and diazepam with 8.5 mg/kg and 0.2 mg/kg dosage, respectively was administered intravenously for anesthesia induction. Anesthesia was continued by inhalation of isoflurane in oxygen following intubation.
Oral cavity was rinsed with 1.2% chlorhexidine, and infiltration anesthesia was administered using lidocaine plus epinephrine. Cervical cavities were prepared in nine maxillary and mandibular incisor teeth using an inverted conical bur. As soon as the pink shadow of the pulp tissue was observed through a thin layer of dentin, the teeth were isolated with cotton rolls and the pulp chamber was exposed using a #2 dental explorer. The cavities were rinsed with saline. Cervical cavities were prepared in a total of 36 incisor teeth (Figure 2). The teeth were then randomly divided into three groups. In the control group, no pulp capping agent was placed

Table 1

Degree of inflammation in the control, MTA and PRF groups

inflammation Degree Group	0×	1××	2×××	3××××	Total
Control	0	2 (16.07%)	5 (41.07%)	5 (41.07%)	12 (100%)
MTA	7 (58.03%)	4 (33.03%)	1 (8.03%)	0	12 (100%)
PRF	3 (25.00%)	9 (75.00%)	0	0	12 (100%)
Total	10 (27.08%)	15 (41.07%)	6 (16.07%)	5 (13.09%)	36 (100%)

× *Absence of inflammatory cells*
×× *Mild inflammation*
××× *Moderate inflammation*
×××× *Severe inflammation*

on the exposure site. In groups 2 and 3, MTA and PRF were applied on the exposure site, respectively. Then entire cavity in all groups, was restored with Zonalin (Zinc oxide eugenol, Purton, Wiltshin, Sweden) (Figure 3). The teeth were evaluated in terms of discoloration and inflammation on a weekly basis.

After 2 months, the teeth in all three groups were extracted under general anesthesia (13). The teeth were fixed in 10% buffered formalin, decalcified and subjected to histological analysis. Tissue sections were embedded in paraffin blocks and stained with hematoxylin and eosin (14). The stained slides were observed under a light microscope at x10 and x40 magnifications. One pathologist observed the slides and scored them according to the degree of inflammation, formation of hard tissue and dentinal bridge and occurrence of necrosis. The pathologist was blinded to the group allocation of specimens and type of pulp capping agent used. Inflammation was scored as follow.

0: Absence of inflammatory cells

1: Small number of neutrophils and mononuclear inflammatory cells

2: Moderate infiltration of inflammatory cells, neutrophils and leukocytes

3: Severe infiltration of inflammatory cells, neutrophils and leukocytes such that they occupied more than two/thirds of the pulp chamber.

Hard tissue and dentinal bridge formation in the samples was scored as follow.

0: No formation of dentinal bridge

1: Slight deposition of hard tissue beneath and at the margins of the pulp capping agent

2: Moderate deposition of hard tissue beneath and at the margins of the pulp capping agent

Presence of denatured and autolyzed proteins in the pulp tissue indicated the presence of necrosis.

Statistical analysis

Data were analyzed using SPSS version 21. The frequency and percentage of scores for degree of inflammation, formation of dentinal bridge and presence/absence of necrosis in the three groups were calculated and reported. The non-parametric Kruskal-Wallis test was used to compare the three groups in terms of degree of inflammation, dentinal bridge formation and presence/absence of necrosis. Pairwise comparisons were carried out using non-parametric Mann Whitney U test. Considering the significance of the topic, the mean and standard deviation of degree of inflammation and dentinal bridge formation scores were separately calculated

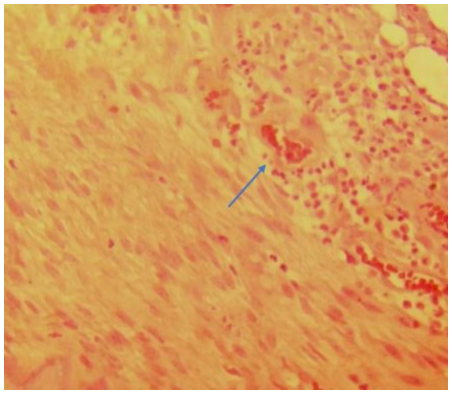


Figure 4
Moderate inflammation in the PRF group.

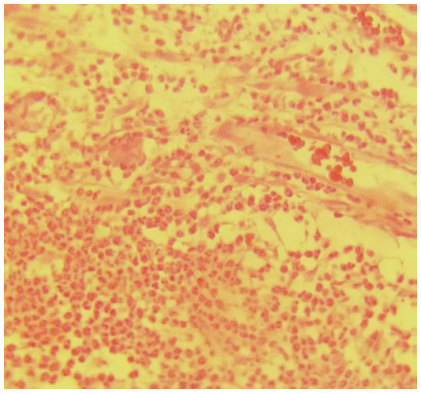


Figure 5
Severe inflammation and pulp necrosis in the control group (x40 magnification).

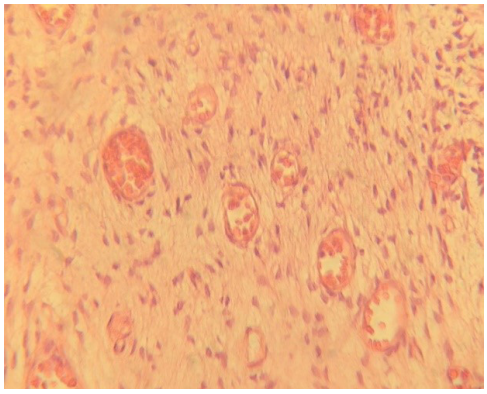


Figure 6
Angiogenesis and fibroplasia in the PRF group (x40 magnification).

in the control and PRF groups and were compared using the Mann Whitney U test. Level of significance was set at 0.05.

Results

Inflammation

Three groups were significantly different in terms of degree of inflammation. Significant differences were noted between the MTA and control (P<0.0001) and also between the PRF and control (P<0.002) groups in terms of degree of inflammation but the difference between the MTA and PRF groups (P=1.0) was not significant in this regard.

Table 1 shows the degree of inflammation in the three groups. Figures 4 to 6 indicate different degrees of inflammation and angiogenesis in the PRF and control groups.

Dentinal bridge formation

The results showed that the MTA and PRF groups had equal performance with regard to hard tissue and dentinal bridge formation (P=1) when used as pulp capping agent and their efficacy was significantly higher than that of the control group (P=0.0001). Table 2 shows the degree of hard tissue

Table 2

Hard tissue and dentinal bridge formation in the control, MTA and PRF groups

Dentinal bridge formation Group	0×	1××	2×××	Total
Control	12 (100%)	0	0	12 (100%)
MTA	0	4 (33.03%)	8 (66.03%)	12 (100%)
PRF	0	4 (33.03%)	8 (66.07%)	12 (100%)
Total	12 (33.03%)	8 (22.02%)	16 (44.04%)	36 (100%)

× *No dentinal bridge formation*
×× *Slight deposition of hard tissue*
××× *Moderate deposition of hard tissue*

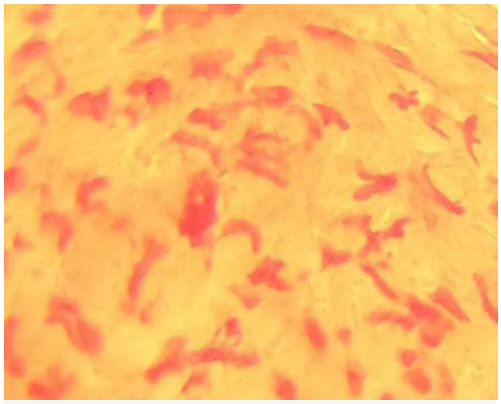


Figure 7
No dentinal bridge formation in pulp in the control group (x10 magnification).

Figure 8
Islands of deposited hard tissue (initiation of dentinal bridge formation) in the MTA group (x40 magnification).

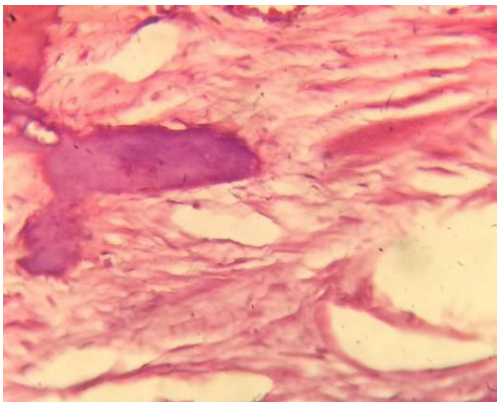
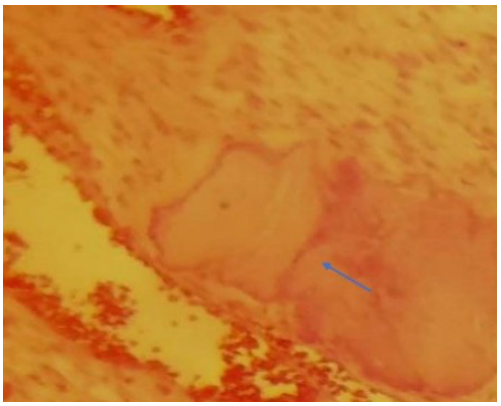


Figure 9
Thick dentinal bridge formation in the PRF group (x40 magnification).



and dentinal bridge formation in the three groups. Figures 7 to 9 show hard tissue and dentinal bridge formation in different groups.

Necrosis

MTA and PRF had equal efficacy (P=1) in prevention of necrosis when applied as pulp capping agents and their efficacy was significantly higher than the control group (P=0.0001). Table 3 shows presence/absence of necrosis in the three groups. Figure 10 shows necrosis in the control group.

Table 3

Presence/absence of necrosis in the control, MTA and PRF groups

Necrosis Group	absence×	presence××	Total
Control	0	12 (100%)	12 (100%)
MTA	12 (100%)	0	12 (100%)
PRF	10 (83.03%)	2 (16.07%)	12 (100%)
Total	22 (63.01%)	14 (38.09%)	36 (100%)

× *Absence of necrosis*
xx Presence of denatured and autolyzed proteins in the pulp tissue

Discussion

In the recent years, attempts have been made to find a material to directly cap the exposed vital pulp and enhance regeneration (1). In this study, different histological parameters (dentinal bridge formation, degree of inflammation and presence of necrosis) were compared to assess the efficacy of MTA and PRF for pulp capping in dogs.

In this study, the incisor teeth of dogs were selected because the anatomy of dog teeth has some similarities to that of human teeth. Thus, they are commonly used in experimental models, yielding favorable results (15).

The current results showed that inflammation occurred in all groups; however, degree of inflammation in the control group was higher than that in the MTA and PRF groups. The MTA and PRF groups showed almost equal degrees of pulpal inflammation. Also, dentinal bridge formation was the same in both MTA and PRF groups and significantly higher than that of the control group.

The ability of MTA in dentinal bridge formation is related to its excellent sealing ability and polymerization. Thus, when applied as a pulp capping agent, it is not disseminated into the adjacent tissues and decreases subsequent microleakage (16).

MTA releases hydroxyl and calcium ions when in contact with water and tissue fluids and can induce proliferation of pulp fibroblasts. Takita et al. (2006) evaluated the effects of MTA and calcium hydroxide on human dental pulp stem cells in vitro and showed that MTA, compared with the control group, induced cell proliferation within 12 days. However, calcium hydroxide did not show such results (17). The optimal properties of MTA are responsible for its favorable results when used as a DPC agent. These properties include insolubility, minimal toxicity, excellent marginal

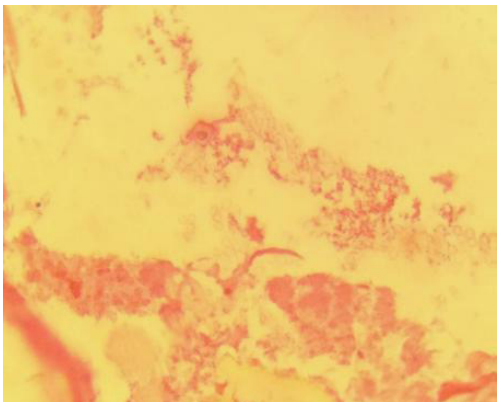


Figure 10 Complete pulp necrosis in the control group (x40 magnification).

adaptation, high setting pH after 3-4 hours and induction of cytokine production by human osteoblasts (16). Shi et al. (2016) evaluated the pulpal reactions to DPC with MTA and another biomaterial in dog teeth and showed that the majority of teeth in both groups demonstrated evidence of calcified bridge formation and did not have pulpal inflammation (18). These observations are in agreement with our results regarding MTA.

Li et al. (2015) in a meta-analysis introduced MTA as a suitable alternative to calcium hydroxide for DPC considering insignificant inflammatory reactions, formation of dentinal bridge and high success rate (19).

Considering all the above and the current results, MTA seems to bring about optimal outcomes when used for DPC. Comparison of PRF and MTA in our study had adequate methodological reliability and considering the similar histological findings in the PRF and MTA groups, it may be concluded that PRF can be used for DPC.

PRF is a non-homogenous biomaterial, on which cells are cultured. It contains plasma, cytokines, leukocytes and different proteins trapped in a very dense fibrin membrane. The fibrin matrix has significant effects on differentiation of osteoblasts (20). Also, growth factors/PRP fibrin matrix may be responsible for dual actions of osteoblasts. Huang et al. (2010) evaluated the biological effects of PRF on human dental pulp cells based on histological observations and showed that PRF stimulates the proliferation and differentiation of human dental pulp cells by up-regulation and expression of alkaline phosphatase (21).

Moreover, PRF can cause controlled release of growth factors over time such that the level of tumor growth factor B1 and platelet-derived growth factor-AB increases to day 14 and decreases afterwards. PRF contains a dense fibrin matrix along with leukocytes, cytokines, glycoproteins and growth factors. Leukocytes in PRF are mainly responsible for the release of growth factors and anti-inflammatory activity (22). This explains the presence of moderate inflammation following DPC with PRF.

Pathak et al. (2014) confirmed the clinical and radiographic success of pulpotomy with PRF in immature human permanent molars in a case report (23). This finding was in agreement with our results. Yang et al. (2013) evaluated the effects of PRF on proliferation and chemotaxis of autogenous dental pulp cells and evaluated the results following its application as a DPC agent in vital pulp therapy. They reported that PRF was biocompatible with human dental pulp cells and use of adequate concentration of PRF exudate enhanced the proliferation and migration of pulp cells. These parameters play a role in pulp healing in vital pulp therapy (24).

In a case report, Lee et al. (2013) reported that pulpotomy with PRF is a biocompatible treatment and PRF in direct contact with the pulp tissue can enhance root development due to its growth factor content (25).

Hiremath et al. (2012) showed that PRF serves as a physiological structure and supports root development. In total, pulpotomy with PRF can replace pulpotomy with MTA or other biomaterials in permanent molars with pulpitis (26).

The current results revealed that dentinal bridge did not form in any of the control samples due to high degree of inflammation. Moreover, PRF and MTA showed similar degrees of dentinal bridge formation. Evidence shows that growth factors present in PRF are released within 1 to 4 weeks after the application of PRF.

The regenerative properties of PRF are evident in its application as a DPC agent.

Also, the expected time for pulp regeneration is the first 3 weeks following application, which was also reported in our study (27).

Thus, observation of reparative dentin in pulp tissue is a sign of pulpal regeneration, which was reported in the MTA and PRF groups after DPC in our study. Huang et al. (2010) demonstrated that PRF can induce proliferation of human dental pulp cells, increase the expression of osteoprotegerin proteins and increase the alkaline phosphatase activity. Thus, in presence of a small number of vital pulp cells, odontoblast-like cells are produced and dentin-pulp complex is formed (28). On the other hand, Wang et al. (2010) showed that cells present in dental pulp may be stem cells and play a role in regeneration of dental pulp (29). Similar results were reported in our study.

Dentinal bridge formation is imperative for pulp vitality. Reparative dentin is formed in the pulpal remnants. Thus, in absence of bleeding, PRF provides growth factors and a potential network for pulp regeneration (27). The present study showed mild to moderate inflammation and slight to moderate deposition of hard tissue beneath and at the margins of the pulp capping agent in incisor teeth of dogs following DPC with PRF and MTA.

Dentinal bridge formation at the interface of pulp tissue-DPC agent is a debated topic because presence of dentinal bridge does not necessarily indicate a healthy pulp status and does not protect the pulp tissue against bacterial penetration. However, it may be a sign of recovery or reaction to stimulation. Dentinal bridge was not formed in any of the control samples in our study. On the other hand, dentinal bridge formation has been noted in exposed pulps without applying a biomaterial (30).

The extracellular matrix on the surface of wound is comprised of healing connective tissue, which is physiologically formed in exposed pulp tissues following deposition of osteodentin or fibrodentin. In this region, odontoblast-like cells produce reparative dentin and eventually show evidence of normal pulp function (31).

Since dental pulp has adequate viable tissue, it seems that DPC treatments can be successful in asymptomatic pulp exposures. A clinical study stated that teeth with asymptomatic pulp exposures remain vital for averagely 12 years following DPC treatment (32).

The efficacy of PRF for enhancement of wound healing has been evaluated and confirmed in different tissues. Growth factors are highly important in signaling, formation and regeneration of dentin-pulp complex. On the other hand, recapitulation of these procedures may lead to pulpal regeneration. Also, micron-scale angiogenesis in PRF fibrin network causes cell migration (26).

As expected, the exposed and uncapped pulp tissue led to necrosis in the control group. Necrosis was also noted in the MTA and PRF groups in lower rate. This can be due to organization of inflammation and pulpal infection following inflammation. If pulp tissue inflammation overcomes the existing infection, subsequent regeneration would occur. Since infection was limited in the MTA and PRF groups, the inflammation overcame it and regeneration occurred in the pulp tissue.

In the present study, PRF was prepared using blood collected from dogs and applied at the site of pulpal exposure. The process of PRF preparation is simple and fast and it does not require activation by bovine thrombin as does the PRP. On the other hand, PRF is an autologous material and risk of transmission of infection and other diseases is insignificant compared with the use of other allografts, xenografts and biomaterials used for DPC (26, 33).

Further in vitro and clinical studies are required to accurately determine the mechanism of action of PRF in pulp tissue regeneration. In vitro studies are highly effective to determine the biological effects of PRF; however, generalization of their results is limited since they cannot well simulate the clinical setting. Future studies are required to assess the effects of PRF and MTA on expression of differentiating odontoblastic markers and dentin matrix proteins.

Conclusions

Comparison of the effects of treatment with MTA and PRF when used as DPC agents in incisor teeth of dogs based on histopathological observations revealed that MTA and PRF both had equal performance with regard to degree of inflammation, dentinal bridge formation and necrosis when used as DPC agents and were superior to the control group.
Considering the optimally equal results of application of PRF and MTA as DPC agents, and easy preparation of PRF compared with PRP, it seems that PRF can bring about optimal results when used for DPC. However, long-term clinical studies are required on this topic.

Clinical Relevance

Knowledge about tissue responses to different pulp capping materials is essential for improving the outcome of direct pulp cap (DPC) treatment. Clinical success of DPC results in more longevity of tooth.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

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Ethical considerations

The study protocol was in line with the Declaration of Helsinki regarding the care and use of laboratory animals. Animal's rights were followed and no unnecessary harm was done to animals.

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