Original Article

Histologic evaluation of vital pulp therapy using propolis and platelet-rich plasma (PRP): Evidence from a canine model

Maryam Gharechahi[1], Saeed Moradi[2], Samar Fatemi[3]

Abstract

Objective: The present study evaluated the pulp tissue response following direct pulp capping (DPC) with several materials in dogs' teeth.

Methods: This study was performed on four doges with 64 mature healthy premolars and molars. The teeth randomly received the following materials: platelet-rich plasma (PRP), propolis, MTA, and glass-ionomer cement (GIC, negative control group). Afterwards, the teeth were restored with light cure GIC. Half of the animals were sacrificed after seven and half after 30 days. Histologic samples were prepared (n=8 per material/interval), and the levels of inflammation, and fibrous and calcified tissue formation were compared between the groups and intervals.

Results: After seven days, inflammation and calcified tissue levels were comparable among the groups ($P > 0.05$). The MTA group showed significantly more fibrous tissue formation than the GIC samples ($P<0.05$). After 30 days, propolis and MTA showed significantly lower inflammation than GIC ($P<0.05$). PRP and MTA groups showed significantly higher fibrous tissue formation than the GIC group ($P<0.05$). Moreover, GIC showed the lowest calcification level among the groups, and PRP had lower calcification than MTA ($P<0.05$). Inflammation levels of the experimental groups decreased after 30 days, but the change was not significant ($P>0.05$). Fibrous tissue formation in the MTA and propolis groups decreased significantly after 30 days ($P<0.05$). Calcification levels of the experimental groups increased significantly over time ($P<0.05$).

Conclusions: The findings suggest that propolis might be a favorable substitute for MTA in DPC procedures, as it produced comparable results to MTA in reducing inflammation and enhancing calcification.

Keywords: Animal model, Dental pulp capping, Histology, MTA, Propolis, Platelet-rich plasma

Introduction

Vital pulp therapy aims to maintain pulp tissue health when it has been compromised by caries, trauma, or restorative dental procedures (1). Various materials have been employed as pulp capping agents. Traditionally, several compositions containing calcium hydroxide have been used, and after that, MTA has been adopted as a direct pulp capping (DPC) agent (2). Dentinal bridge formation beneath the capping agent is one of the favorable pulp responses to capping materials (3, 4). Although using MTA has successfully formed dentin bridges, it has certain disadvantages, such as difficult handling characteristics, long setting time, tooth Discoloration, and relatively high cost (4). Accordingly, various materials have been proposed as possible pulp capping agents, such as propolis and platelet-rich plasma (PRP).

Propolis is a honeybee resin that has been used for centuries in traditional medicine as an anti-inflammatory and antibacterial agent. Of all the constituents of propolis, flavonoids ensure different effects, such as regulating the immune response, decreasing the release of free radicals, and preventing the growth of bacteria and fungi (5, 6). PRP is a rich source of growth factors used in different dentistry fields. PRP has demonstrated good tissue compatibility and hard tissue induction ability (7).

The present study aimed to evaluate the amount of pulp inflammation and fibrous and calcified tissue formation following DPC with MTA, propolis, PRP, and glass ionomer cement (GIC) at 7- and 30-day intervals. The null hypothesis was that there is no significant difference in histologic findings, including the inflammation levels, fibrous tissue formation, and calcified tissue formation, after DPC with MTA, propolis, PRP, and GIC in dogs' teeth.

Materials and Methods

This interventional animal study was carried out in the Animal Research Center of Mashhad Dental School,

[1]Dental Materials Research Center, School of Dentistry, Mashhad University of Medical Sciences, Mashhad, Iran.
[2]Dental Research Center, School of Dentistry, Mashhad University of Medical Sciences, Mashhad, Iran.
[3]Private Practice, Mashhad, Iran.

Corresponding Author: Maryam Gharechahi
Dental Materials Research Center, School of Dentistry, Mashhad University of Medical Sciences, Vakilabad Boulv, Mashhad, Iran.
Email: *gharechahim@mums.ac.ir*

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Mashhad University of Medical Sciences, Mashhad, Iran. The study protocol was approved by the ethics committee and the Research Council of Mashhad University of Medical Sciences (ID 910043).

Sample collection

Four 2-year-old Iranian healthy dogs were obtained from a municipal animal collection site and used in this study. Sixty-four mature and intact teeth (28 molars and 36 premolars) were included in this study. The dogs were kept in the Animal Center of Mashhad Dental School, in separate cages with optimum ventilation, temperature, hygienic standards, nutritious meals, and 12 h light/dark cycle. The animals had the opportunity to adapt to the housing and diet for two weeks before the operation. In this period, vaccinations were carried out with polyvalent and rabies vaccines (Biocan, Bioveta, Czech). All animals were fed twice a day with a soft diet and ad libitum water during the whole experiment.

Pre-operative periapical radiographs were taken under general anesthesia. Two dogs were sacrificed after 7 days and two after 30 days. In each group of animals (n=32), the teeth were randomly divided into four groups using a randomized treatment table (n=8). The PRP, propolis, and MTA were considered experimental groups, and GIC was the negative control group.

Intervention

Thirty minutes before the procedures, 1.0 mL of intramuscular diazepam (Darou Pakhsh, Tehran, Iran) was injected for sedation, followed by the intramuscular injection of 10 mg/kg of anesthetic agent ketamine HCL (Rotex Medica, Germany) and 1 mg/kg of xylazine (Rotex Medica).

After induction of general anesthesia, 10 mL of blood samples were taken from each animal. Next, citrate was added to blood samples at a 1:9 ratio to prevent clot formation. The samples were then sent for preparation of the PRP (8).

The surface of each tooth was cleaned with pumice paste (Kemdent, Swindon, Wiltshire, UK), and isolation was accomplished using a rubber dam. A diamond #14 fissure bur (Jota AG, Rüthi, SG, Switzerland) installed in a high-speed handpiece was used to prepare the endodontic access cavity. A pulpal exposure point was created in the central pit of the tooth. A sterile cotton pellet impregnated with 5.25% NaOCl was placed on the exposed pulp for hemostasis.

Each tooth received the pulp cap material according to its relevant group. Propolis was provided from the beehives in Hezar Masjed Mountains, Iran, and it was processed at the Razi Institute Research Center in Mashhad, Iran. Every 1 mL of water-based propolis contains 7-10 mg of the effective material (flavonoids). In the MTA group, ProRoot MTA (Dentsply, Tulsa Dental, Tulsa, Oklahoma, USA) was mixed according to the manufacturer's instructions and placed on the exposure site. In the PRP group, the jelly PRP was injected into the cavities up to the level of the cementoenamel junction and left to form a clot.

After pulp capping in all teeth, sterile parafilm was placed over the capping materials. All the cavities were restored with light-cure GIC (Fuji IILC, GC Corporation, Tokyo, Japan). An experienced endodontist performed the procedures. Dogs were kept on a soft diet for ten days postoperatively. During this period, they received Carprofen (Rimadyl tablet®, Zoetis, USA) as analgesics at a dose of 4.4 mg/kg once daily.

During the post-treatment periods (7 and 30 days after pulp-capping), the animals were sacrificed with a lethal intravenous overdose of sodium pentobarbital (2 dogs at seven days and two dogs at 30 days). The maxilla and mandible were dissected and reduced in volume. Subsequently, the pulp-capped teeth were removed in blocks (tooth/bone) using a water-cooled diamond disk and radiographed.

Histologic examination

The teeth were extracted and fixed in 10% formalin for ten days, followed by immersion in normal saline for one day. Next, the teeth were placed for 6 months in a decalcifier (Agitator, Lip Shaw MFG Co, Detroit, USA), which was an agitator containing 17% ethylene diamine tetra acetic acid (EDTA) (Asia Chemi Teb. Co., Tehran, Iran). During this time, EDTA was refreshed every other day; afterwards, the teeth were placed in refined ethyl alcohol with increasing concentrations of 70%, 80%, 90%, and 95%. Then, the samples were embedded in paraffin wax, and 5 µm thick sections were prepared. Finally, the sections were stained using the hematoxylin and eosin (H & E) staining method for histological evaluation.

The degree of inflammation was classified as normal pulp structure, mild inflammation (increased capillary, fibroblast, and inflammatory cells and few extravasated red blood cells), moderate inflammation (more inflammatory cells and increased capillary and vessels), severe inflammation (significant cellular infiltration, excessive blood vessels), and pulp necrosis (9).

Fibrosis was scored as 0 (0%), 1 (1%-30), 2 (30%-60%), or 3 (>60%), according to the related surface covering.

Calcification was scored as 1 (≤ 0.1 mm), 2 (0.1-0.25 mm), or 3 (≥ 0.25 mm), based on the dentinal bridge thickness (10).

Statistical analysis

Data analysis was conducted using SPSS software version 23.0 (IBM Corp., Armonk, NY, USA). The histologic findings of the samples between 7- and 30-day intervals were compared using the Mann-Whitney U test. The Kruskal-Wallis test was used to compare the histologic findings among the different groups. Mann-Whitney U test was run for pairwise comparisons between the two groups. The significance level was established at $P<0.05$.

Results

In the MTA group, scattered calcification was observed in some areas after seven days (Figure 1A), whereas dentin bridge formation was seen after 30 days (Figure 1B).

In the PRP group, the tissue response was severe fibrosis and moderate inflammation on day seven (Figure 2A). After 30 days, a fibrous matrix was noticed in some areas, and calcification was seen as separate calcified masses (Figure 2B).

Fibrous tissue, scattered calcifications, and mild inflammation were observed in the propolis group after seven days (Figure 3A). After 30 days, partial dentinal bridge formation and calcification were noticed (Figure 3B).

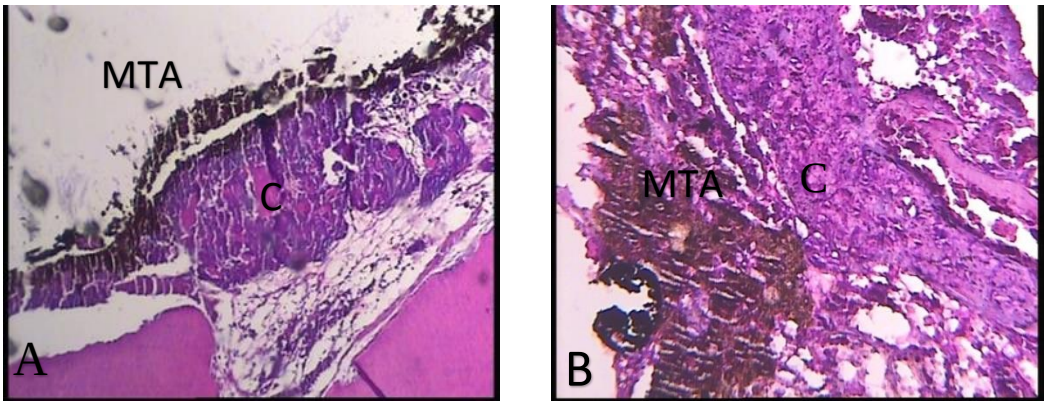

Figure 1. Light microscopy views (H&E staining; x 40) after direct pulp capping with MTA at intervals of 7 (A) and 30 (B) days. Calcified tissue (C) is formed at the pulp tissue and capping material interface.

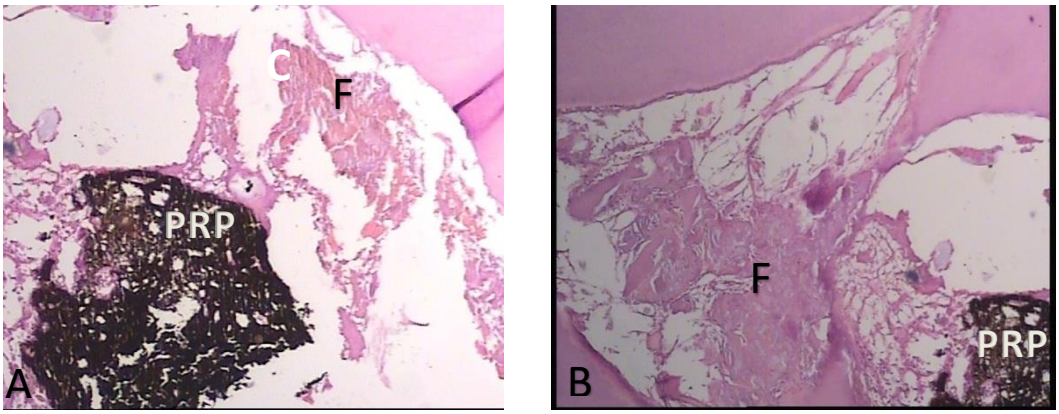

Figure 2. Light microscopy views (H&E staining; x 40) of samples after direct pulp capping with PRP at intervals of 7 (A) and 30 (B) days after treatment. The formation of calcified (C) and fibrous tissues (F) is evident at the pulp tissue and capping material interface.

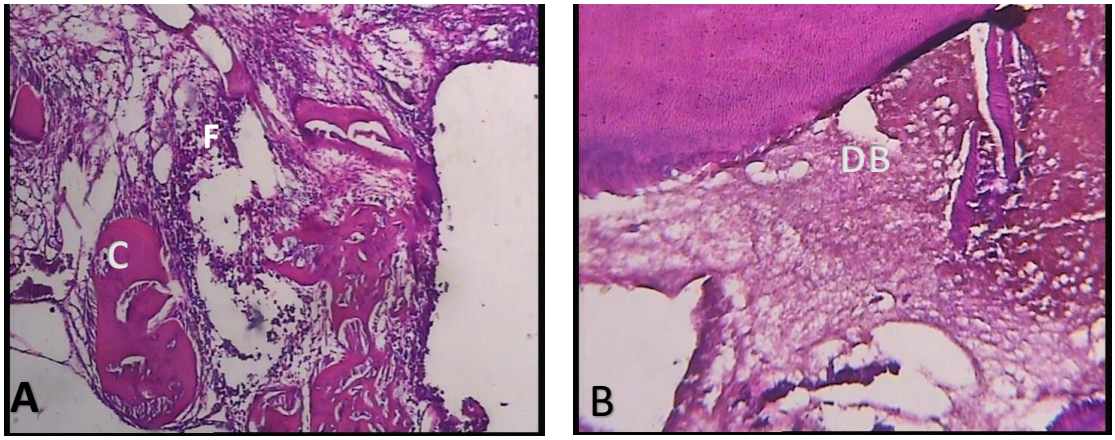

Figure 3. Light microscopy views (H&E staining; x 40) of samples after direct pulp capping with propolis at intervals of 7 (A) and 30 (B) days after treatment. The formation of calcified tissue (C), partial dentinal bridge (DB), and fibrous tissue (F) is evident at the pulp-capping material interface.

Histologic findings after seven days

Table 1 represents the histologic findings in the samples in each group after seven days. According to the result of the Kruskal-Wallis test, no statistically significant difference was observed between the groups regarding the inflammation level (P=0.883) and calcified tissue formation (P=0.26). There was a significant difference in fibrous tissue formation among the groups (P=0.036; Table 1). The amount of fibrous tissue in the propolis and PRP groups was higher than in the GIC, but the difference was insignificant (P>0.05). However, using MTA led to a significantly higher fibrous tissue formation than GIC samples (P<0.05).

Histologic findings after 30 days

As illustrated in Table 1, the average amount of inflammation (P=0.009), fibrous (P=0.009) and calcified (P<0.001) tissue formation was significantly different between groups after 30 days. Inflammation in the MTA and propolis groups was mild and significantly lower than that in the GIG group (P< 0.05). The average amount of fibrous tissue in the PRP and MTA groups was significantly greater than that in the GIG group (P< 0.05).

Calcified tissue formation in the GIG group was significantly lower than that in the other groups (P<0.05).

Table1. Comparison of the histologic findings between groups in the 7th and 30th days.

Histologic finding	Group	7 days		30 days		P-value
		Mean ± SD	Median	Mean ± SD	Median	
Inflammation	MTA	2.67 ± 0.52	3	1.83 ± 0.75[a]	2	0.057
	Propolis	2.50 ± 0.55	2.5	1.83 ± 0.75[a]	2	0.116
	PRP	2.67 ± 0.52	3	2.33 ± 1.03[a,b]	2	0.388
	GIC	2.50 ± 0.55	2.5	3.67 ± 0.52[b]	4	0.011*
	P-value	0.883		0.009*		
Fibrous tissue formation	MTA	3.5 ± 0.55[a]	3.5	2.5 ± 0.55[a]	2.5	0.019*
	Propolis	3.17 ± 0.75[a,b]	3	2 ± 0.63[a,b]	2	0.025*
	PRP	3.0 ± 0.63[a,b]	3	2.83 ± 0.75[a]	3	0.434
	GIC	2.17 ± 0.75[b]	2	1.33 ± 0.52[b]	1	0.057
	P-value	0.036*		0.009*		
Calcification	MTA	1.33 ± 0.52	1	3.83 ± 0.41[a]	4	0.002*
	Propolis	1.17 ± 0.41	1	3.33 ± 0.82[a,b]	3.5	0.003*
	PRP	1.0 ± 0.0	1	2.67 ± 0.52[b]	3	0.002*
	GIC	1.0 ± 0.0	1	1.0 ± 0.0[c]	1	1.00
	P-value	0.26		<0.001*		

*P-values less than 0.05 represent a significant difference between the 7th and 30th-day intervals or among the groups.
Different superscripted letters in each histologic category represent a statistically significant difference between the groups.
MTA= mineral trioxide aggregate; GIC= glass-ionomer cement; PRP= platelet-rich plasma

Calcified tissue formation in the propolis and MTA groups was comparable (P>0.05), but the PRP group showed a significantly lower calcification level than the MTA group (P<0.05).

Comparison of 7-day and 30-day intervals

Table 1 illustrates the difference in histologic findings between groups at 7- and 30-day intervals. The incidence of inflammation after pulp capping with MTA, Propolis, and PRP was lower in the samples obtained on the 30th day compared to the 7th day, but the difference was not statistically significant (P>0.05; Table 1). In contrast, the inflammation level increased significantly in the GIC group after 30 days (P=0.011; Table 1). In the GIC group, 70% of the samples had an accumulation of chronic inflammatory cells beneath the capping area (severe inflammation), and necrotic areas were found in some regions. This group did not exhibit odontoblastic differentiation or dentinal bridge formation**.**

The fibrous tissue production after pulp capping was significantly reduced in the MTA (P=0.019) and propolis (P=0.025) groups after 30 days (Table 1). The difference in fibrous tissue formation between the intervals was insignificant in the samples capped with GIC (P=0.057) and PRP (P=0.434) materials (Table 1).

Calcified tissue formation increased significantly on the 30th day compared to the 7th day in the MTA (P=0.002), propolis (P=0.003), and PRP (P=0.002) groups; however, it did not change significantly in the GIC group (P=1.0; Table 1).

Discussion

The findings of the current animal study revealed a significant difference between the groups regarding the histological findings and time intervals; therefore, the null hypothesis was rejected**.**

Glass-ionomer cement (GIC) was applied as the negative control in this study. This selection was based on earlier research demonstrating the lack of differentiation of odontoblast-like cells following DPC with GIC (11, 12). Dentinal bridge formation through tertiary dentinogenesis is a primary goal of conservative pulp therapy, typically occurring one month following the surgical procedure. The development of pulp healing areas depends highly on the employed pulp capping agents (13).

In the cases of DPC with MTA and propolis, gradual increases in calcified tissue formation and decreases in fibrous tissue were observed over time. After 30 days, the average amount of inflammation in the MTA and propolis groups was significantly lower than in the GIC group, and dentin bridge formation was detectable in both groups, with no statistically significant difference between them**.** Propolis and PRP yielded similar results regarding calcification and dentinal bridge formation. However, the calcification level achieved with PRP was significantly lower than that of the MTA material. The average amount of inflammation in the PRP group did not significantly differ from that of the other materials including GIC. These findings imply that propolis might be a favorable substitute for MTA in DPC treatments, as it produced comparable results to MTA in reducing inflammation and enhancing calcification, potentially aiding tissue regeneration.

MTA possesses several favorable characteristics that render it a valuable material for DPC in permanent adult teeth. Previous studies have demonstrated positive short-term outcomes when using MTA for partial or complete pulpotomy or DPC in human subjects (11, 12, 14, 15). Other noteworthy properties of MTA include maintaining an alkaline pH after curing, excellent sealing capabilities, the gradual release of calcium ions, and the unique capacity to induce dental pulp regeneration. Consequently, MTA was considered the gold standard for DPC in the current study. Despite the high success rates reported with MTA, limitations such as the high cost, technique sensitivity, and difficult re-entry into the canal may hinder its routine use (17). Further pulp therapy in teeth capped with MTA can be challenging due to the chance of canal obliteration.

Propolis can stimulate the formation of TGF-β1, essential for the differentiation of odontoblasts and promoting the synthesis of collagen by pulp cells. The success of propolis is often attributed to its antibacterial and anti-inflammatory properties and its compatibility with biological systems. It also appears to facilitate the formation of hard tissue dentin bridges, making it a valuable component in DPC and pulpotomy procedures (18).

The outcomes of this study are consistent with several investigations (19-25). Some studies demonstrated the construction of thick dentine bridges, accompanied by well-organized odontoblast-like cells when propolis was used (19, 20). Meto et al. (21) found that Albanian propolis had a significant regenerative effect and enhanced dentin barrier formation in a piglet model 3 months after pulpotomy. Likitpongpipat et al. (22) suggested that propolis extract might be a promising

alternative for pulp capping procedures. This is because when applied to mechanically exposed pulp, it stimulates reparative dentine formation with organized dentinal tubules. Parolia et al. (23) compared the effects of MTA, propolis, and Dycal in direct pulp capping. Their findings indicated that propolis-induced pulp responses were similar to those caused by MTA. Furthermore, Kantrong et al. (24) demonstrated that propolis extract exhibits an anti-inflammatory effect by reducing COX-2 induction and enhancing PGE2 synthesis when dental pulp cells are exposed to IL-1β treatment. This anti-inflammatory potential was also observed in the present study since inflammation in pulps capped with MTA and propolis was similar to each other and significantly lower than the GIC group after 30 days. Moradi et al. (25) observed a significant elevation in the expression of the tenasin marker in pulps capped with propolis after 30 days. This marker induces odontoblast differentiation and is associated with dentinogenesis.

PRP is another well-known substance used for different treatment procedures in dentistry and medicine. PRP is a suspension of growth factors (GF) that are naturally found in platelets. These GFs, such as platelet-derived growth factor (PDGF) and TGF-β, play a crucial role in wound healing and tissue regeneration (25-28). In the current study, it was observed that the formation of dentinal bridge (calcified tissue) in the PRP group was not significantly different from that in the propolis group but significantly lower than that of the MTA group.

Ohshima et al. (27) conducted a study to assess the impact of PRP on human pulp cells and found that the formation of a hard tissue barrier was observed in pulp cells adjacent to PRP in the 4- and 8-week samples. They concluded that PRP exhibits good compatibility with tissue and has the potential to stimulate the development of hard tissues**.** Mansour et al. (29) reported a better result than that obtained in this study. They mixed direct pulp capping materials with platelet-rich fibrin (PRF) and observed dentinal tubules in most hard tissue barriers (29). It should be noted that in this study, we utilized PRP, in contrast to Mansour et al. (29), who employed PRF. The three-dimensional nanofiber structure of PRF enables a gradual release of growth factors over up to 10 days, whereas PRP releases a significant amount of proteins within the first hour. Furthermore, PRF can serve as a scaffold or resorbable membrane, facilitating the delivery of cell lineages to damaged areas (24). Furthermore, PRP comprises a low-density fibrin network and necessitates the addition of anticoagulants as well as a two-step centrifugation process. In contrast, PRF is characterized by a high-density fibrin network and can be prepared using a single-step centrifugation process without the requirement for anticoagulants (30). One limitation associated with PRP is the technical sensitivity of the multiple steps involved in its preparation, which increases the risk of preparation errors.

When the timeframe was extended from 7 days to 30 days, there was a decrease in inflammation observed in MTA, propolis, and PRP groups, although this decrease was not statistically significant. Notably, fibrosis levels in the MTA and propolis groups exhibited a significant reduction during this period. Furthermore, there was a significant increase in calcified tissue formation following pulp capping with MTA, propolis, and PRP**.**

From a histological perspective, the MTA group showed the development of a relatively thin dentinal bridge. In the propolis group, the formation of the bridge appeared incomplete. In the PRP group, calcification presented a scattered distribution.

The limitation of this study was the small sample size in the groups. To obtain a more comprehensive understanding of the quality of the hard tissue barrier formed after pulp capping with PRP and propolis, it is recommended to conduct similar studies with extended observation periods and larger sample sizes**.** Furthermore, Platelet-Rich Fibrin (PRF) is suggested for further investigation in the context of pulp capping studies. Lastly, considering the potential variations between animal and human responses, conducting this study with human samples may provide valuable insights.

Conclusions

According to the result of the current study:

1- Propolis and MTA showed significantly lower inflammation levels than the negative control group at 30 days.
2- After 30 days, all experimental groups (MTA, propolis, and PRP) showed significantly higher calcification levels than the negative control group. The PRP group showed a significantly lower calcification level than the MTA group.
3- Altogether, propolis might be a favorable substitute for MTA in DPC treatments, as it produced comparable results to MTA in reducing inflammation and enhancing calcification, potentially aiding tissue regeneration.

Conflicts of interest

The authors deny any conflicts of interest related to this study.

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