A comparative histological analysis of human pulp following direct pulp capping with Propolis, mineral trioxide aggregate and Dycal

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ABSTRACT

Background: Permanent teeth pulp exposures have traditionally been treated with calcium hydroxide pulp capping. The aim of this study was to investigate the response of human pulp tissue which were mechanically exposed to a new material, Propolis and compare it with two existing and commonly used pulp capping agents (mineral trioxide aggregate and Dycal).

Methods: Thirty-six intact human premolars were mechanically exposed. Teeth were divided into six groups of 6 teeth each and were capped with Propolis, mineral trioxide aggregate and Dycal. Final restoration was done with posterior composite resin using light cured glass ionomer cement as a liner. The teeth were then extracted on the 15th or the 45th day and processed for histological evaluation.

Results: Differences in inflammatory response and dentine bridge formation of the exposed pulp to the three different materials were statistically calculated using chi-square test and were found to be non-significant. There was more pulp inflammation in teeth treated with Dycal than with Propolis and MTA on the 15th as well as on the 45th day. Propolis and MTA showed bridge formation in more teeth, and the bridges were in closer proximity to pulp capping material than teeth treated with Dycal on the 45th day.

Conclusions: The response of pulps to Propolis as a pulp capping agent was comparable to MTA and Dycal.

Keywords: Pulp capping, Propolis, mineral trioxide aggregate, Dycal, dentine bridge.

Abbreviation: MTA = mineral trioxide aggregate.

(INTRODUCTION

The foundation of restorative dentistry rests on the principle that the maintenance of a healthy and functional pulp-dentine complex would result in the successful healing of the exposed pulp. Like other connective tissue, pulp tissue has the potential to heal. Characteristics of the healing exposed pulp tissue include reorganization of damaged soft tissue, differentiation of odontoblast-like cells from subodontoblast cells and repair of the exposed dentine tissue with reparative dentine bridge formation. Factors that may compromise pulp healing include bacterial penetration through the restoration-tooth interface, cytotoxicity of dental materials, sensitivity of operative procedures and status of the pulp. Direct pulp capping involves the placement of a biocompatible agent on pulp tissue that has been inadvertently exposed from traumatic injury or by iatrogenic means. The objectives of the treatment are to seal the pulp against bacterial penetration, to encourage the pulp to wall off the exposure site by initiating a dentine bridge and to maintain healthy pulp tissue.

A wide range of materials have been suggested for the dressing of the exposed pulp. Many studies have indicated that calcium hydroxide compounds are the gold standard for pulp capping in human teeth. However, some of the limitations reported with calcium hydroxide compounds include the presence of tunnels in the dentine barrier, extensive dentine formation obliterating the pulp chamber, high solubility in oral fluids, lack of adhesion and degradation after acid etching.

As a result of these limitations, a variety of materials have recently been proposed as candidates for direct pulp capping; one such material is mineral trioxide aggregate...
aggregate (MTA). Initially, MTA was used in endodontics to seal off all the pathways of communication between the root canal system and the external surface of the tooth.\cite{13}

Pitt Ford et al.\cite{14} were the first to evaluate the performance of MTA for pulp capping in monkey teeth, and their study illustrated the superior performance of MTA in comparison to calcium hydroxide. Although several case reports and clinical studies have evaluated the effect of MTA for pulp capping in permanent human teeth,\cite{15,16} only a few histologic studies have been conducted to evaluate the histologic response of MTA in human teeth.\cite{17}

Recently, a natural product, Propolis (Russian penicillin) has shown to possess potent antimicrobial and anti-inflammatory properties.\cite{18,19} This resinosus substance, varying in colour from yellow-brown to dark-brown, is collected from trees and shrubs by honeybees. The main chemical classes present in Propolis are flavonoids, phenolics and other various aromatic compounds. Flavonoids are well-known plant compounds which have antioxidant, antibacterial, antifungal, antiviral and anti-inflammatory proprieties. Propolis as an anti-inflammatory agent has shown to inhibit synthesis of prostaglandins. It also supports the immune system by promoting phagocytic activities, stimulating cellular immunity and augmenting healing effects. Additionally, it contains elements such as iron and zinc which are important for the synthesis of collagen.\cite{20,21}

Hence, the purpose of this clinical study was to evaluate the histological response of healthy pulp to honey comb extract (Propolis) when used as a direct pulp capping agent and to compare it with MTA and calcium hydroxide (Dycal).

MATERIALS AND METHODS

The investigation protocol was approved by the Institutional Ethical Committee of Manipal Academy of Higher Education. Consent forms were signed by patients and parents after receiving a detailed explanation about the experimental rationale, clinical procedures and possible risks associated with the study. Thirty-six healthy human premolar teeth scheduled to be extracted for orthodontic reasons were selected from patients ranging from 15 to 25 years old. All teeth were examined clinically and radiographically to assure the absence of caries, trauma, periapical and periodontal lesions. The status of the pulp was assessed by using heat test, cold test and electric pulp test (Digitest, Parkell pulp vitality tester; NY, USA).

The procedure commenced with the administration of local anaesthetic. Teeth were cleaned using 0.2% chlorhexidine solution. After rubber dam application, occlusal cavities were prepared by means of sterile diamond burs (No. 330 Diatech) at high speed under air-water spray coolant. The prepared occlusal cavities were 3.0 to 3.5 mm in occlusal depth, 4.0 to 4.5 mm in mesiodistal width and 3.0 to 3.5 mm in faciolingual width. Dimensions of the cavity were checked using a digital caliper in an attempt to standardize the cavity size. Pulp exposure was performed in the centre of the pulp floor by means of a sterilized round diamond bur (ISO 010 Diamond-Horico) under water cooling. One bur was used for each cavity. Complete haemostasis was achieved by applying gentle pressure on the exposed site with cotton pellet moistened with sterile saline. The cotton pellets were renewed till the complete haemostasis was established and the exposure site was dried with sterile dry cotton pellet. The teeth were then divided into six experimental groups (n = 6) (Table 1).

After haemostasis, the exposure site was dressed with pulp capping materials directly in contact with the pulp tissue.

In Groups I and II, 100% Propolis powder (Ecuadorean Rainforest LLC, USA) was mixed with 70% ethyl alcohol to a thick consistency on a paper pad with the aid of plastic spatulas. The mixture was carried to the exposure site in a metal carrier.

In Groups III and IV, ProRoot MTA (Dentsply Caulk Milford, DE, USA) powder was mixed according to the manufacturer’s instructions. The mixture was carried to the exposure site in a metal carrier.

In Groups V and VI, Dycal (Dentsply Caulk Milford, DE, USA) base and catalyst were mixed on a paper pad with plastic spatulas according to the manufacturer’s instructions. The mixture was carried to the exposure site using a Dycal carrier.

After placement of the pulp capping materials, teeth were restored with composite resin (MN Z100, 3M ESPE, St Paul, MN, USA) using resin modified glass ionomer cement (GC Fuji II (LC) GC Corporation Tokyo, Japan) as a liner.

Table 1. Experimental groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>DPC Material</th>
<th>Teeth were extracted after</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Propolis</td>
<td>After 15 days</td>
</tr>
<tr>
<td>II</td>
<td>Propolis</td>
<td>After 45 days</td>
</tr>
<tr>
<td>III</td>
<td>MTA</td>
<td>After 15 days</td>
</tr>
<tr>
<td>IV</td>
<td>MTA</td>
<td>After 45 days</td>
</tr>
<tr>
<td>V</td>
<td>Dycal</td>
<td>After 15 days</td>
</tr>
<tr>
<td>VI</td>
<td>Dycal</td>
<td>After 45 days</td>
</tr>
</tbody>
</table>

Patients belonging to Groups I, III and V were asked about the presence of postoperative sensitivity or pain over 15 days, while patients belonging to Groups II, IV and VI were asked the same over 45 days. Teeth from Groups I, III and V were extracted after 15 days, whereas teeth from Groups II, IV and VI were extracted after 45 days under local anaesthesia. After extraction, the apical third of all teeth were sectioned to facilitate fixation in 10% formalin (Qualigen’s GL, India.
Limited). After this teeth were demineralized in Good-
ing Stewart Fluid (Formal Formic Acid; Thomas Baker
Private Ltd., Mumbai, India – 90% formic acid 100 ml,
formalin 50 ml, distilled water 850 ml), later washed in
distilled water and then dehydrated in ascending grades
of N-butyl alcohol and embedded paraffin. Serial
sections of 6 μm were cut using soft tissue microtome
(Reichart Zung 2040) in a bucco-lingual plane. It was
then placed on gelatin-coated slides and stained with
haematoxylin and eosin. The sections were blindly
evaluated by experienced pathologists and calibrated
according to the criteria described as follows:22

**Inflammatory cell response**

Grade 1. Absent or very few inflammatory cells.

Grade 2. Mild or average number less than 10 inflamm-
atory cells.

Grade 3. Severe inflammatory lesion appearing as an
abscess or dense infiltrate involving one-third
or more of the coronal pulp.

Grade 4. Completely necrotic pulp.

**Dentine bridge formation**

Grade 1. Presence of a dentine bridge directly adjacent
to some portion of the medicament interface.

Grade 2. Presence of a dentine bridge distant from the
medicament interface.

Grade 3. No evidence of any dentine bridge formation
in any sections.

The scores attributed for each group were subjected to
chi-square test. A p value of <0.05 was established to
state the statistically significantly difference (Tables 2
and 3).

**RESULTS**

**Histomorphologic features**

**Inflammatory response on the 15th day**

In Group I 33%, in group III 83% and in group V 17%
of the specimens exhibited very few and scattered
inflammatory cells (polymorphonuclear leukocytes or
mononuclear lymphocytes) in the pulp, whereas 67% in
Group I, 17% in Group III and 83% of the specimens in
Group V showed moderate inflammatory infiltrate
(Fig 1).

**Inflammatory response on the 45th day**

In both Groups II and IV, 83% of the specimens and in
Group VI 33% of the specimens exhibited very few and scattered
inflammatory cells in the pulp, while 17% of
the specimens in both Groups II and IV, and 67% of
specimens in Group VI showed moderate inflammatory
infiltrate.

Differences in the inflammatory response of three
different materials were statistically calculated using
chi-square test and were found to be non-significant
on the 15th day (p = 0.054) and on the 45th day
(p = 0.105).

**Hard tissue bridge on 15th day**

In Group I 33% (Fig 2), in Group III 67% (Fig 3) and
in Group V 33% of the specimens exhibited hard tissue
bridges.

<p>| Table 2. Statistical analysis of samples showing inflammatory cell response |</p>
<table>
<thead>
<tr>
<th>Days</th>
<th>Materials</th>
<th>Inflammatory cell response scores</th>
<th>Statistical comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>15</td>
<td>Propolis</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>MTA</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Dycal</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>45</td>
<td>Propolis</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>MTA</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Dycal</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

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Hard tissue bridge on 45th day

On the 45th day, 6 teeth (100%) in both Groups II (Fig 4) and IV (Fig 5) and 5 teeth (83%) in Group VI showed dentine bridge formation (Fig 6) which was more continuous and thicker than that observed in the 15th day specimens.

In the Propolis and MTA group, four specimens showed a well-organized odontoblast-like cell layer formed adjacent to the dentinal bridge with tubular dentine. In the remaining specimens, odontoblast-like cells were found adjacent to the dentinal bridge with irregular pattern of tubules. Whereas in the Dycal group only two specimens showed an odontoblast-like cell layer with irregular tubular dentine adjacent to the dentinal bridge. The remaining samples showed amorphous and a non-tubular calcific bridge exhibiting a dense structure.

Differences in dentine bridge formation of the three different materials were statistically calculated using chi-square test and were found to be non-significant on the 15th day (p = 0.592) and on the 45th day (p = 0.539).

DISCUSSION

Knowledge of pulp physiology has now broadened, leading to a better understanding of the conditions which are necessary for the pulp to heal. The criteria
that characterize a successful direct pulp capping vary among authors. They generally agree that pulp capping is clinically successful if the tooth functions free of symptoms, reacts adequately to sensibility tests and has a normal radiographic appearance. However, the clinical criterion is inadequate for an evaluation of the long-term prognosis for teeth treated by pulp capping. It is impossible to clinically diagnose teeth in which healing is complicated by inflammation. Therefore, a critical evaluation of the results of pulp capping can only be made histologically.23

In the present study, specimens capped with Propolis exhibited less inflammation on both the 15th day and 45th day. This could be related to the anti-inflammatory property of Propolis. Flavonoids and caffeic acid present in Propolis are known to play an important role in reducing the inflammatory response by inhibiting the lipoxygenase pathway of arachidonic acid. Flavonoids and caffeic acid also aid the immune system by promoting phagocytic activities and stimulating cellular immunity. The stimulation of various enzyme systems, cell metabolism, circulation and collagen formation could contribute to the hard tissue bridge formation by Propolis. These effects have been shown to be the result of the presence of arginine, vitamin C, provitamin A, B complex and trace minerals such as copper, iron, zinc as well as bioflavonoids.24 All these factors assist in faster healing of the wound. In addition to its wound healing ability, Propolis is a good antimicrobial agent. It breaks down bacterial cell wall, cytoplasm and prevents bacterial cell division.25

In the present study, Dycal was used as a conventional pulp capping material which has been a gold standard. Dycal with its lower pH may avoid major tissue damage and directly stimulate reparative dentine.26 Most of the Dycal specimens in the present study showed incomplete bridge formation with more inflammation of the pulp tissue. In one specimen, black particles of the capping material were detected within the phagocytes. This finding may suggest that the material is gradually dissolved. A recent study suggested that Dycal should be substituted with MTA because the latter showed less inflammation and more predictable results after pulp capping.27 Various studies have reported excellent results for MTA when used as a pulp capping material.28,29 The results of the present study confirmed the favourable outcome of this material when compared to Dycal in terms of lack of inflammation and better quality of bridge formation. Studies have shown that MTA and calcium hydroxide have a similar mechanism for hard tissue formation.26,28,30 Several authors have reported favourable properties of MTA such as high alkalinity (that has been related to its bactericidal properties),31 excellent seal32,33 and biocompatibility.34,35

In the present study, Propolis showed no statistically significant difference in pulp response when compared to Dycal and MTA as a pulp capping agent. This could be due to the presence of a good seal, which is a critical factor for the success of the pulp capping procedure. There is no general agreement on whether the toxicity of material placed on vital pulp tissue or the material’s ability to seal the cavity from further bacterial ingress determines a successful outcome. It could possibly be a combination of multiple factors. The effect of various dental materials on the prognosis of pulp exposure has been evaluated in several studies.27,36–39 It is generally accepted that accidental pulp exposure offers more favourable prognosis when compared to carious pulp exposure.40 Even the control of bleeding is a critical factor for the success of pulp capping. According to studies, sodium hypochlorite in concentrations of 0.25% was therapeutically efficacious without being toxic. But since sodium hypochlorite has a pH of 12, it may extract growth factors from the walls of dentine just as calcium hydroxide.1 Thus, in the present study, the therapeutic influence of pulp capping material was observed in the induction of reparative dentinogenesis, free of any supplementary chemicals or products which can mimic such an action. For this reason saline was used to control haemorrhage.41 The present study was carried out under ideal conditions where pulps were healthy when exposed. So the pulp response to any procedure or material was likely to be different to that of a compromised pulp as a result of caries, cracks or breakdown of restorations that allow bacteria to penetrate the tooth and irritate the pulp. Hence, a careful consideration is essential to adapt these favourable results in clinical situations.

CONCLUSIONS

The response of pulp to Propolis as a pulp capping agent was comparable to MTA and Dycal.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the assistance and support of Mrs. Alkka Manuel who reviewed the original manuscript.

REFERENCES


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