Radiographic and histopathologic outcomes of immature dog teeth with apical periodontitis after revascularization using Propolis. Abdelsalam et al.
Histopathological pulp response of dog’s teeth capped with biosealer and biodentine: An in vivo study

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Abstract

Introduction: The aim of this study was to evaluate the pulpal response after pulp capping using either biodentine (BD) or tech biosealer capping (TBC) in the dog model.

Materials and Methods: Class V cavities were carried out on 45 teeth in three mongrel dogs. The dental pulp was exposed in 30 teeth (2 experimental groups) and left unexposed in 15 teeth (control group). The cavities of the experimental groups were capped with either BD (n = 15 teeth) or TBC (n = 15 teeth). All cavities in the experimental and control groups were restored with resin-modified glass ionomer. Dentin bridge formation, architecture of the odontoblastic layers, and signs of inflammation were assessed after 1, 2, and 3 months using the computer image analyzer.

Results: The BD group exhibited a thick newly formed reparative dentin bridge completely closing the exposure site with cell inclusions and mineralization, variable numbers of odontoblast-like cells, preserved pulp tissue, marked numerous collagen fibers, and blood vessels. While the TBC group exhibited an incomplete newly formed reparative dentin bridge with tunnel defect, vacuolated odontoblasts, complete pulp degeneration with multiple edematous spaces, hyperemic blood vessels, extravasated red blood cells, multiple calcified structures scattered just beneath the dentin bridge and through the pulp tissue, and newly ill-defined odontoblasts.

Conclusion: For pulp capping, BD has a better dentin bridge formation and pulp preservation than TBC in the dog model.

Keywords: Calcium silicate, dental pulp, dentin bridge, odontoblasts, pulp capping

INTRODUCTION

Preservation of the pulp vitality after traumatic, carious, or iatrogenic injuries is a challenge. Several biomaterials have been used for direct pulp capping with various degrees of success. The prognosis of direct pulp capping depends upon several factors such as sealing ability and biocompatibility of the pulp capping material and the ability of the pulp to heal after injury.[1]
For several decades, calcium hydroxide has been considered the “gold standard” for direct pulp capping materials.\[2\] However, it has several drawbacks such as high solubility, poor sealing ability to dentin, and formation of multiple tunnel defects in the dentin bridge adjacent to the material.\[3\]

Portland cements such as proRoot mineral trioxide aggregates (MTA) have been used for pulp capping.\[4‑8\] Compared to calcium hydroxide, MTA stimulates a faster and thicker dentin bridge formation. However, the MTA has some disadvantages such as poor handling characteristics, expensiveness, and delayed setting time.\[6‑8\]

Therefore, several materials have been developed to overcome the aforementioned disadvantages of both calcium hydroxide and MTA.\[9,10\] A calcium silicate-based cement called Biodentine (BD) has been developed in 2011.\[11\] The BD cement has high density, low porosity, fast setting time (12 min), good biocompatibility, positive effect on the vital pulp cells, and the ability to enhance the reparative and tertiary dentin formation.\[12,13\] In addition, the BD has some physical and mechanical advantages over the calcium hydroxide such as low porosity, high compressive strength, low solubility, high density, and high-sealing ability to dentin.\[13\]

Calcium silicate-based cements have received increasing attention due to their high biocompatibility and adequate biological response obtained in both laboratory and clinical investigations.\[14,15\] Therefore, a new calcium silicate-based pulp capping material called Tech Biosealer Capping (TBC) was introduced to the market.

According to the manufacturer’s instructions, TBC (TBC, Isasan SRL, Revello Porro, Italy) can be used for vital pulp therapy.

The pulp response to the available pulp capping materials may vary, depending upon the properties of the pulp capping material. Therefore, the use of a new material must be based upon the laboratory and experimental studies. For this reason, there was an interest to increase the knowledge about the properties of both BD and TBC. Hence, this study evaluates the pulpal response after pulp capping using either BD or TBC in the dog model.

**MATERIALS AND METHODS**

Two commercial materials were used in the present study; BD (Septodont, Saint-Maur-des-Fossés, France) and TBC material.

**Ethical approval**

All international and institutional guidelines for animal use and care were followed up. The protocol of this study was approved by the Ethical Committee at Faculty of Dental Medicine for Girls, Al-Azhar University, Cairo, Egypt (15-12-14, Dent. Biomat.).

**Animals**

Three healthy mongrel dogs weighting about 15–20 kg and aged 1–2 years were selected for this study. These dogs were purchased commercially from the Al-Fahad Trading Company of Animals (Abu-Rawash, Giza, Egypt). The dogs were examined and kept under observation in separate cages (1.5 m × 2.5 m × 3 m) for 2 weeks before their using as experimental animals in the study. They were kept under the good conditions of ventilation, nutrition, cleaning, and 12 h light/dark cycle. The animals were given two meals of soft food daily and clean water \textit{ad libitum}.

**Classification of the teeth**

Fifteen teeth in each dog, including incisors, canines, and premolars were used, summing up 45 teeth. These teeth were randomly divided according to the treatment protocol into three equal groups:

- Group I (control group-15 teeth): Class V cavities were performed without exposure of the dental pulps
- Group II (BD group-15 teeth): The dental pulps were exposed and capped with BD
- Group III (TBC group-15 teeth) The dental pulps were exposed and capped with TBC.

All groups were represented in each dog. Each group was further subdivided into three subgroups (five teeth each) according to the post-treatment evaluation period: Subgroup 1 (1 month), Subgroup 2 (2 months), and Subgroup 3 (3 months).

**Procedures**

After fasting the dogs for 12 h, general anesthesia was administrated. The dogs were premedicated with subcutaneous injection of atropine sulphate at a dose of 0.05 mg/kg and intramuscular injection of xylazine HCl at a dose of 1 mg/kg. The anesthesia was induced by intravenous administration of ketamine HCl at a dose of 5 mg/kg through a cannula fixed in the cephalic vein. The anesthesia was maintained by intravenous injection of 2.5% thiopental sodium solution at a dose of 25 mg/kg (dose to effect).

The teeth were disinfected by povidone-iodine solution. A dry field was achieved by the cotton rolls and gauze swabs. Class V cavities were performed at the buccal surfaces of teeth, approximately 2 mm coronal to the gingival margin.
using a #2 round carbide bur (SS White, Rio de Janeiro, Brazil) at a low speed under copious sterile normal saline solution. Deepening of each cavity was continued until the appearance of pulpal shadow. In the control group, the dental pulps were left unexposed. In the experimental groups, the pulp was exposed by a probe, and the pulpal exposures were standardized to 1 mm in diameter. The bleeding was controlled by rinsing the exposure site with sterile saline solution. The cavities of the experimental groups were randomly divided into two groups as follows:

In Group (II), the exposed pulps were capped with BD. Both BD powder and liquid were mixed according to manufacturer’s recommendations in an automatic mixture (amalgamator) for 30 s. The putty-like mixture was dispensed on a mixing pad and applied to the cavity by an amalgam carrier.

In Group (III), the exposed pulps were capped with TBC. The powder was mixed with the liquid to produce a homogenous paste according to the manufacturer’s instructions. This paste was applied to the exposure site by an amalgam carrier, and a moist cotton pellet was then placed over the TBC.

The remained cavity of all experimental teeth and whole cavities of the control teeth were filled with resin-modified glass ionomer (GC Corporation, Tokyo, Japan).

**Histopathologic evaluation**

According to the posttreatment evaluation period, the dogs were sacrificed by overdose of general anesthetic solution (20 mL Thiopental sodium 5% solution) injected quickly through the cephalic vein. The jaws were separated and bone segments (blocks), including the experimental and control teeth were resected. The bone blocks were fixed in 10% buffered formalin solution with a ratio of 1:50. After 2 weeks of fixation, the samples were decalcified using 17% ethylenediaminetetraacetic acid (EDTA) solution with pH 7. The decalcifying solution was renewed on a daily basis for about 150 days. Perforation of the specimens was carried out by a fine needle to allow the penetration of the EDTA solution. The specimens were examined weekly for decalcification. After decalcification, the samples were dehydrated as usual and embedded in paraffin blocks. The blocks were sectioned in a bucco-lingual plane at 6 μm thickness. Sections were stained using hematoxylin and eosin (H and E) for histopathologic evaluation.

The stained sections were assessed by the image analysis software Image J 1.41 (NIH, Bethesda, Maryland, USA). Photomicrographs were captured by a digital camera attached to the light microscope by a C-mount. The magnification of the photos captured for analysis was fixed at (×40, 100 and 200). The pulp response to the tested pulp capping materials along the posttreatment evaluation periods was evaluated. The histological changes of the pulp tissues, including dentin bridge formation, architecture of the odontoblastic layers, and signs of inflammation were assessed.

**RESULTS**

**Control group**

At all evaluation periods, the control group exhibited a normal histological pulp architecture consisting of normal connective tissue. The odontoblasts at the lateral wall of the pulp showed normal and uninterrupted palisading arrangement. Continuous regular layers of reparative dentin were separated from the primary dentin by a line of demarcation [Figure 1]. No signs of inflammation were noticed in the pulp.

**Biodentine group**

**Dentin bridge formation**

At all evaluation periods, a thick newly formed reparative dentin bridge was seen. This dentin bridge completely closed the exposure site. Variable amounts of cell inclusions (odontoblast-like cells) and blood vessels were observed inside the dentin bridge giving the appearance of both osteo and vasodentin, respectively. A continuous reparative dentin was also observed with variable thickness along the lateral wall of the pulp. A line of demarcation was seen between the primary and the reparative dentin [Figure 2a]. In addition, a layer of predentin was observed [Figure 2b].

**Figure 1:** (a) A photomicrograph of the control group showing the morphological aspects of the pulp (P) beneath the cavity resembling the normal histological architecture of the pulp tissue (H and E, ×40). (b) A photomicrograph of the control group showing the primary dentin (D), regular and continuous reparative dentin extending along the lateral walls of the pulp (green arrow), line of demarcation (black arrow), predentin (yellow arrow), and odontoblastic layer (H and E, ×200)
**Pulp tissue**
In Subgroups 1 and 2, a preserved pulp tissue marked proliferating odontoblastic layers, marked numerous collagen fibers, and mild inflammation were observed [Figures 3a and b]. In Subgroup 3, minimal changes were observed after 3 months such as concentrated collagen fibres and congested blood vessels.

**Odontoblasts**
At all evaluation periods, proliferating odontoblasts appeared as multiple successive layers with no signs of inflammation [Figures 4a and b]. After 3 months, a mature tall odontoblastic layer was observed along the lateral surface of the dentin [Figure 4c].

**Tech biosealer capping group**

**Dentin bridge formation**
After 1 month, a thick incomplete reparative dentin bridge was seen and nearly closed the exposure site [Figure 5]. A tunnel defect was observed near to the capping material. A layer of reparative dentin was also seen and extended along the lateral wall of the pulp. There was a line of demarcation between the primary and the reparative dentin.

After 2 months, multiple calcified structures (bone or dentin-like structures) were scattered just beneath the dentin bridge and through the pulp tissue [Figure 6]. The predentin and reparative dentin were also observed.

After 3 months, a newly formed reparative dentin bridge was seen and completely closed the exposure site. The predentin layer and reparative dentin were also seen in the lateral root canal wall.

**Pulp tissue**
After 1 month, complete degeneration of the pulp tissue with multiple edematous spaces was seen [Figure 5]. There were several vacuole-like spaces with various shapes and sizes, hyperemic blood vessels, extravasated red blood cells (RBCs), and multiple inflammatory cell infiltrates.

After 2 and 3 months, the pulp had multiple calcified structures, loose connective tissue, and multiple hypermic blood vessels [Figures 6 and 7].

**Odontoblasts**
After 1 month, the cells in the odontoblastic layer were vacuolated along the lateral wall. After 2 and 3 months, a newly formed continuous odontoblastic layer was seen. The odontoblasts had ill-defined boundaries [Figure 7].

**DISCUSSION**
Pulp capping materials protect the vital pulp after exposure due to removal of deep carious lesions or trauma. For several decades, conventional or resin-modified calcium hydroxide/oxide–based materials have been applied for direct pulp capping due to the release of calcium and hydroxyl ions.\(^{[16]}\)

Nowadays, calcium silicate-based cements such as MTA and BD are commonly used as pulp capping materials. Recently, another new calcium silicate-based materials called biosealer is introduced to the market. To our
knowledge, there are no in vivo studies on biosealer as pulp capping agent. Therefore, this study compared both BD and biosealer as the direct pulp-capping materials. In the present study, the BD was selected for comparison due to its excellent sealing ability.\(^{[17]}\) Moreover, there are several in vivo studies on the MTA.\(^{[6,7,9]}\)

Both animal and human teeth are suitable to demonstrate the effects of pulp capping materials on vital pulp tissue.\(^{[18]}\) Therefore, the dogs were enrolled in this study as an animal model because the mechanism of reparative dentinogenesis is similar to that of humans, but in a short time. In addition, the dog has a suitable pulp size for the histopathological evaluation and a good number of teeth allowing the comparison of several pulp capping cement in the same dog.\(^{[19]}\)
Like several previous studies, a direct pulp capping technique was used in this study to induce the formation of reparative dentin at the injury site. The formation of dentin bridge was considered as a sign of the success of pulp capping material.

BD became commercially available as a dentin replacement cement in 2009. TBC is another calcium silicate-based product which is similar to the MTA cement. Up to the authors, knowledge, it is not yet used as a direct pulp capping material. Although it is necessary to use a dental amalgamator for the preparation of the BD, no additional tools are needed for the Tech Biosealer preparation. However, the results of the present study showed better therapeutic effects of BD than TBC as direct pulp capping materials regarding the dentin bridge formation and pulp integrity preservation.

In the present study, Class V cavities were selected for easy handling of the materials and protection from occlusal forces. Furthermore, mechanical perforation of the cavity floor with a probe was used to expose the pulps. This technique was recommended by several authors because it protects the pulp from extensive damage and creates a uniform pulp exposure. Although this technique may lead to pushing of the dentin fragments into the pulp, these fragments did not induce an inflammatory pulpal response. In addition, the auto-induction of reparative dentinogenesis may be observed on the surfaces of these fragments.

In this study, the evaluation depended upon the histopathological changes elicited with the pulp capping procedure and the detection of dentin bridge formation, which are essential criteria for monitoring of the healing process. Formation of the dentinal bridge at the interface between the pulp and pulp-capping cement is a controversial issue because it may be a reaction to irritation or a sign of healing. In the present study, the formation of the dentinal bridge was interpreted as a positive reaction to stimulation and a sign of healing according to the histological findings.

In the current study, both BD and TBC cement induced early reparative dentinogenesis because of the physicochemical properties of these materials that enhance the mineralization process. Moreover, the stimulation of cell proliferation and differentiation might be attributed to the tricalcium silicate present in the tested materials and the presence of both calcium and silicon ions. Similar explanation was mentioned before.

After 1 month, the dentin bridge was completed in the BD group while it was considered complete, but with a tunnel defect in TBC group. This tunnel defect was regarded as an undesirable site, facilitating the migration of microorganisms toward the pulp. This favorable therapeutic action of BD cement might be attributed to a significant release of transforming growth factor-β1 in the pulp cells that stimulates the odontoblasts to increase their activity and enhances the reparative dentinogenesis. Similar findings were reported in several previous studies.

In the TBC group, vacuole-like spaces, complete degeneration of the pulp tissue, multiple edematous spaces, hyperemic blood vessels extravasated RBCs, and vacuolated odontoblastic layer were observed after 1 month. These findings might be due to the difference in the cytotoxic effect between the BD and TBC. The BD had significantly less cytotoxic effect compared to the TBC. This difference may be due to the specific chemical compositions of these cement, and it requires more research in future.

In the BD group, the dentin bridge with normal pulp was observed in all teeth after 2 months. In most teeth of the BD group, odontoblasts were arranged just below the dentin bridge with some structural changes. These cells were not true odontoblasts, but odontoblast-like cells having elongated shape and palisade orientation. These findings are consistent with other studies. Odontoblast-like cells produce extracellular matrix that becomes a complete dentin bridge after mineralization. The thickness of the dentin bridge and the pulp preservation depends upon the amount of odontoblast-like cells. With increased layers of these cells, the thickness of dentin bridge is increasing, and the pulp remains vital.

After 3 months, normal pulp tissues, layers of odontoblasts with normal architecture and arrangement, predentin, secondary reparative dentin were detected in the BD group. These findings are in agreement with several studies. While in the TBC group, predentin layer, reparative dentin and new odontoblasts were recorded. These findings were considered as an improvement in the pulp healing process after the findings at 2 months where the odontoblasts had ill-defined boundaries. This improvement might be due to the decrease of cytotoxic effects of the TBC with time. Finally, the results of this biological study are in concurrence with the chemical-physical and mechanical properties of the BD and TBC.

The main limitations of this study were the small sample size used and the relatively short time of evaluation.
Therefore, this study suggests more extended investigations on a large sample size to address the specifications and influences of the TBC on the dental pulp cells and their reparative capabilities to form the dentin bridge.

Under the circumstances of this study, BD has a better therapeutic outcome than the TBC after the pulp capping procedure. Therefore, further studies are recommended to evaluate the cytotoxicity of the TBC and to assess its clinical application as a pulp capping material in the human.

CONCLUSION

The BD has a better dentin bridge formation and pulp preservation than TBC after direct pulp capping in the dog model.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES