Biosealer versus Biodentine for direct pulp capping in dog's teeth: A histopathological evaluation

Inas M. Al-Sherbiny

Al-Azhar University

Mona H. Farid

Al-Azhar University

Ashraf M. Abu-Seida (✉ ashrafseida@cu.edu.eg)

Cairo University Faculty of Veterinary Medicine <https://orcid.org/0000-0001-5466-2016>

Inas Motawea

Al-Azhar University

Hagar A. Bastawy

Al-Azhar University

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Abstract

Background

This study compared the effect of Biodentine (BD) and Tech Biosealer Capping (BS) on the pulpal tissue response after pulp capping in dogs, teeth.

Methods

Three mongrel dogs were enrolled in this study. Class V cavities with pulp exposure were performed on the buccal surface of 30 teeth (2 experimental groups) and left without pulp exposure in 15 teeth (control group). The cavities of experimental groups were capped with either Biodentine (BD Group, N= 15 teeth) or Tech-Biosealer Capping (BS Group, N= 15 teeth). All cavities (experimental and control groups) were restored with resin-modified glass ionomer. Dentine bridge formation, architecture of the odontoblastic layers and signs of inflammation were assessed after 1, 2 and 3 months using the computer image analyzer (Leica Qwin 500).

Results

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

Conclusion

For pulp capping, Biodentine has better dentine bridge formation and pulp preservation than Tech Biosealer Capping.

Background

Preservation of pulp vitality after traumatic, carious or iatrogenic injuries is a challenge. There are several biomaterials used for direct pulp capping with various degrees of success. The prognosis of direct pulp capping depends upon several factors such as; ability of the pulp capping material to prevent bacterial microleakage and its biocompatibility as well as ability of the pulp to heal after injury [1].

For several decades, Calcium hydroxide ($\text{Ca}(\text{OH})_2$) has been considered the "gold standard" of direct pulp capping materials [2]. However, it has several disadvantages such as; dissolving over time, 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]. MTA stimulates faster and thicker dentin bridge formation than Calcium hydroxide. However, MTA also has some disadvantages such as; poor handling characteristics, expensiveness and delayed setting time [6–8].

The researches on direct pulp capping are still continued in order to discover a new ideal pulp capping material. Therefore, several materials were developed to overcome the aforementioned disadvantages of both Calcium hydroxide and MTA [9].

Calcium silicate-based cement called Biodentine has been developed in 2011 by Gilles Richard and Olivier Marie [10]. Compared to MTA, Biodentine has high density, low porosity, fast setting time, good biocompatibility, positive effect on vital pulp cells and ability to enhance reparative dentine and to stimulate tertiary dentin formation [11,12]. In addition, Biodentine has some advantages over Calcium hydroxide such as; decreased porosity, high compressive strength, low solubility, high density and high sealing ability to dentine [12].

Calcium silicate-based cements have received increasing attention due to their high biological compatibility and adequate biological response obtained in both laboratory experiments and clinical cases [13,14]. Therefore, another new calcium silicate-based pulp capping material called Tech Biosealer Capping (TBC) was introduced to the market.

According to the manufacturer's instructions (Tech Biosealer Capping, Isasan SRL, Revello Porro, Italy), TBC can be used for vital pulp therapy. Moreover, the pulp reaction may vary with the use of different available materials, depending upon their properties, which could cause severe damage to the pulp tissue. Therefore, the use of a new material must be based on experimental and laboratory studies.

For this reason, there was an interest to increase the knowledge about properties of both Biodentine and TBC. Hence, this study evaluated the response of dog's dental pulp capped with Biodentine and Tech Biosealer Capping.

Materials And Methods

Three commercial materials were used in the present study. Biodentine, Tech-Biosealer Capping material and Resin-modified glass ionomer. The materials used in the present study regarding their composition, manufacturers and batch number are presented in Table 1.

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 the institute.

Animals:

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

Class V cavities were performed on the buccal surface of the teeth with pulp exposure. These cavities were prepared and capped with Biodentine.

Group II (BS - 15 teeth)

Class V cavities were prepared on the buccal surface of the teeth with pulp exposure and capped with Tech Biosealer Capping.

Group III (Control - 15 teeth)

Class V cavities were prepared on the buccal surface of the teeth without pulp exposure.

All groups were represented in each dog. Each group was further subdivided into 3 subgroups (5 teeth each) according to the post treatment evaluation period into; subgroup 1 (1 month), subgroup 2 (2 months) and subgroup 3 (3 months).

Procedures:

After fasting the dogs for 12h, general anesthesia was administrated.

The dogs were premedicated with Atropine sulphate at a dose of 0.05 mg/kg body weight injected subcutaneously and Xylazine HCl at a dose of 1mg/kg body weight injected intramuscularly. The anesthesia was induced by Ketamine HCl injected intravenously at a dose of 5 mg/kg body weight through a cannula fixed in the cephalic vein. The anesthesia was maintained by Thiopental sodium at a dose of 25 mg/kg body weight 2.5 % solution injected intravenously (dose to effect).

The teeth were disinfected by Povidone iodine solution. A dry field was achieved by means of cotton rolls and gauze swabs. Class V cavities were performed at the buccal surfaces of teeth, approximately 2mm coronal to the gingival margin using a # 2 round carbide bur (SS White, Rio de Janeiro, Brazil) at low speed under copious sterile normal saline solution. Deepening of each cavity was continued until the

appearance of pulpal shadow then the pulp exposures were standardized to 1mm in diameter in both experimental groups. Bleeding was controlled by rinsing the exposure site with sterile saline solution. In control group, the cavities were left without pulp exposure. The cavities of experimental groups were randomly divided into two groups as follows:

Group I (BD group):

The exposed pulps were capped with Biodentine. Both Biodentine powder and liquid were mixed according to manufacturer recommendations in an automatic mixture (amalgamator) for 30 sec. The putty like mixture was dispensed on a mixing pad and was applied to the cavity by amalgam carrier.

Group II (BS group):

The exposed pulps were capped with TBC. The powder was mixed with the liquid to produce a homogenous paste according to the manufacturer instructions. This paste was applied to the exposure site.

The remained cavity of all experimental teeth and whole cavities of control teeth were filled with resin-modified glass ionomer.

Histopathologic evaluation:

The dogs were sacrificed according to the post treatment evaluation period using over dose of general anesthetic solution (20 mL Thiopental sodium 5% solution) injected through the cephalic vein. Jaws were separated and bone segments including the experimental and control teeth were resected.

Blocks were fixed using 10% buffered formalin solution with ratio 1:50. After two weeks of fixation, the samples were decalcified using 17% EDTA solution with pH 7. The decalcifying solution was renewed on daily basis for about 150 days. Perforation of the specimens to allow EDTA penetration was carried out by a fine needle. 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 buccolingual plane at 6µm thickness. Sections were stained using hematoxylin and eosin (H&E) for histopathologic evaluation.

The stained sections were assessed by the image analysis software Image J 1.41 (NIH. 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 (X40, 100, and 200). The pulp response to the tested pulp capping materials along the post treatment evaluation periods was evaluated. The histological changes of the pulp tissues were assessed and included dentin bridge formation, architecture of the odontoblastic layers and signs of inflammation.

Results

Control group:

At all evaluation periods, the pulp tissue showed normal histological architecture consisting of normal connective tissue. The odontoblasts surrounded the lateral wall of the pulp showed normal and uninterrupted palisading arrangement. Continuous regular layers of secondary dentin were separated from the primary dentin by a line of demarcation (Fig. 1).

Biodentine group (BD 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 amount of cell inclusions (odontoblast-like cells) were observed inside the dentin bridge as well as multiple blood vessels 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 also observed between primary and secondary dentin (Fig. 2a). Additionally, a layer of predentin was seen (Fig. 2b).

Pulp tissue:

After one month and two months, preserved pulp tissue with a marked proliferating odontoblastic layer and marked numerous collagen fibers was observed (Figs. 3a&b). Minimal changes were observed after 3 months such as concentrated collagen fibers and congested blood vessels.

Odontoblasts:

At all evaluation periods, proliferating odontoblasts appeared as multiple successive layers with no signs of inflammation (Figs. 4a&b). After three months, mature tall odontoblastic layer was observed along the lateral surface of dentine (Fig. 4c).

Biosealer group (BS group):

Dentin bridge formation:

After one month (Fig. 5), a thick incomplete reparative dentin bridge was seen and nearly closed the exposure site. 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 primary and reparative dentine. After 2 months (Fig. 6), multiple calcified structures (bone or dentin-like structures) were scattered just beneath the dentin bridge and through the pulp tissue. Predentin with reparative dentine was also observed. After 3 months, a newly formed reparative dentine bridge was seen and completely closed the exposure site. Predentin layer and reparative dentine were also seen in the lateral root canal wall.

Pulp tissue:

After one month (Fig. 5), a complete degeneration of the pulp tissue with multiple edematous spaces was seen. There were several vacuole-like spaces with various shapes and sizes, hyperemic blood vessels and extravasated RBCs. After two and three months, the pulp had multiple calcified structures, loose connective tissue and multiple hyperemic blood vessels (Figs. 6&7)

Odontoblasts:

After one month, the cells in the odontoblastic layer were vacuolated along the lateral wall. After two and three months, a newly formed continuous odontoblastic layer was shown. The odontoblasts had ill-defined boundaries (Fig. 7).

Discussion

Pulp capping materials protect the vital pulp tissue after exposure due to deep dental caries or trauma. Most of the available pulp capping materials has several advantages and disadvantages [15, 16]. Therefore the research on new pulp capping materials is still continuing.

Recently, a new calcium silicate-based material called Tech Biosealer Capping is introduced to the market. To our knowledge, there are no *in vivo* studies on Biosealer as a pulp capping agent. Therefore this study compared the efficacy of TBC and Biodentine as direct pulp capping materials. We selected Biodentine for comparison due to its excellent results as pulp capping agent. Moreover, there are several *in vivo* studies on MTA [6,7,9].

Both animal and human teeth are suitable to demonstrate the effects of pulp capping materials on vital pulp tissue [17]. Therefore, dogs were enrolled in this study as an animal model because the mechanism of reparative dentinogenesis is similar to that of human in a short time. Additionally, the dog has a suitable pulp size for the histopathological evaluation and good number of teeth that allow the comparison of several pulp capping cements in the same dog [18]. The used teeth were disinfected with antiseptic solution and isolation with sterile cotton rolls. Application of a rubber dam was difficult due to anatomical consideration of dog's teeth.

Like several previous studies [19,20], direct pulp capping technique was used in our study to induce the formation of reparative dentin at the injury site. The formation of dentine bridge was considered as a sign of success of pulp capping material.

Although both Biodentine and TBC are calcium silicate based products, there are several differences. For example, the dental amalgamator is essential for preparation of Biodentine while no additional tools are needed for TBC preparation. The powder of Biodentine has tricalcium and dicalcium silicate, calcium carbonate and oxide filler, iron oxide shade, and zirconium oxide. The liquid consists of calcium chloride and a hydrosoluble polymer [21]. According to the manufacturer, the powder of TBC consists of a mixture of white Calcium Enriched Mixture (CEM), calcium sulphate, calcium chloride, montmorillonite and the liquid has DPBS.

Mechanical perforation of the cavity floor with a probe was used to expose the pulps in this study. This technique was recommended previously because it protects the pulp from extensive damage and creates a uniform pulp exposure [22]. Although this technique may lead to pushing of dentin fragments into the pulp, these fragments do not induce an inflammatory pulpal response [23]. In addition, the auto-induction of reparative dentinogenesis could be observed on the surfaces of these fragments [24].

In the present study, the evaluation depended upon histopathological changes elicited with the pulp capping procedure and detection of dentine bridge formation, which are essential criteria for monitoring of the healing process.

Our results showed better therapeutic effects of Biodentine than TBC as direct pulp capping materials regarding dentine bridge formation and pulp integrity preservation.

Formation of the dentinal bridge at the interface between the pulp and pulp-capping cement is a controversial issue because it can be a reaction to irritation or a sign of healing [25]. In the present study, formation of dentine bridge was interpreted as a positive reaction to stimulation and a sign of healing according to the histological findings.

In the present study, both Biodentine and TBC cements induced an early reparative dentinogenesis because the physicochemical properties of these materials enhanced the mineralization process. Moreover, stimulation of cell proliferation and differentiation might be attributed to tricalcium silicate present in the tested materials and presence of both calcium and silicon ions. Similar explanation was mentioned in previous studies [26, 27].

After one month, dentine bridge was completed in BD group while it was incomplete with tunnel defect in BS group. This tunnel defect was regarded as undesirable site facilitating the migration of the microorganisms towards the pulp. This favorable therapeutic action of Biodentine cement might be attributed to a significant release of TGF- β 1 in pulp cells that stimulated odontoblasts to increase their activity and enhance the reparative dentinogenesis. Similar findings were reported before [28].

In BS 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 one month. These findings might be due to the difference in cytotoxicity between Biodentine and TBC. Biodentine had significantly less cytotoxic effect compared to TBC [29]. This difference may be due to the specific chemical compositions of these cements and this difference requires more research in the future.

In BD group, dentine bridge was observed in all teeth after 2 months with normal pulp. In most teeth of BD group, odontoblasts were arranged just below the dentine 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 previous studies [30–32]. Odontoblast-like cells produce extracellular matrix that becomes complete dentine bridge after mineralization. The thickness of

dentine bridge and pulp preservation depends upon the amount of odontoblast-like cells. With increased layers of these cells, the thickness of dentine bridge is increasing and the pulp remains vital [33].

After 3 months, normal pulp tissues, layers of odontoblasts with normal architecture and arrangement, predentin, secondary reparative dentin were detected in BD group. These findings are in agreement with several studies [31,33]. While in BS group, predentin layer, reparative dentin and new odontoblasts were recorded. These findings were considered as an improvement in pulp healing process after our findings at 2 months where the odontoblasts had ill-defined boundaries. This improvement might be due to decreasing of cytotoxic effects of Biosealer with time. Therefore further investigations are recommended to evaluate the biocompatibility of TBC.

Conclusion

As a direct pulp capping material, Biodentine has better therapeutic effects than Tech Biosealer Capping in terms of dentine bridge formation and pulp preservation.

Abbreviations

BD: Biodentine.

BS: Bioseler.

TBC: Tech Biosealer Capping.

MTA: Mineral trioxide aggregates.

CEM: Calcium Enriched Mixture.

Table

Table 1. Materials used in the study.

Batch number	Composition	Manufacturer	Materials
310982	Powder: tricalcium silicate Liquid: aqueous calcium chloride solution.	Septodont, Saint-Maur-des-Fossés, France	Biodentine (BD)
E14008	Powder: mixture of white Calcium Enriched Mixture (CEM), calcium sulfate, calcium chloride, montmorillonite Liquid: DPBS (Dulbecco's Phosphate Buffered Saline)	Isasan srl, Rovello Porro, Co, Italy	Tech-Biosealer capping (BS)
707031	Powder: silicate glass powder. Liquid: polyacrylic acid.	GC Corporation, Tokyo, Japan	Resin-modified glass ionomer (Fuji VIII)

Declarations

Ethics approval and consent to participate

This article has no studies with human participants. All international and institutional guidelines for animal use and care were followed. Protocol of the study was approved by the ethical committee at Faculty of Dental Medicine for Girls, Al-Azhar University, Cairo, Egypt.

Consent for publication

Not applicable

Availability of data and materials

All data used and/or analyzed during this research are available from the corresponding author on reasonable request.

Competing interest

The authors declare no conflict of interests

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Authors' contributions

Dr. IMA and Dr. HAB: Animal study and analysis of the data. Prof. MHF and Prof. ITM: planning and supervision of the research project. Prof. AMA: Animal study, writing of the manuscript and supervision of the research. All authors read and approved the final manuscript.

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Figures

Figure 1

(a) The morphological aspects of the pulp (P) beneath the cavity resembling the normal histological architecture of the pulp tissue (H&E×40). (b) A Higher magnification of figure (a) showing primary dentin (D), regular and continuous secondary dentin extending along the lateral walls of the pulp (green arrow), line of demarcation (black arrow), predentine (yellow arrow) and odontoblastic layer (OL) (H&E ×200).

Figure 2

(a) A photomicrograph of group I (Biodentin group) after one month showing the pulp capping material on the exposure site (BD), a thick newly formed reparative dentin bridge completely closing the exposure site (Db), cell inclusions inside the dentin bridge (blue arrow) and multiple blood vessels (yellow arrows). P: Pulp tissue (H&E ×100). (b) A high magnification of Fig. (2a) showing proliferating odontoblasts appeared as multiple successive layers (OL) and numerous collagen fibers (C). Notice the predentin (red arrow), primary dentin (D), reparative dentin (blue arrow) and the line of demarcation (black arrow, H&E ×200).

Figure 3

(a) A photomicrograph of group I (Biodentin group) after two months showing capping material on the exposure site (BD), newly formed reparative dentin bridge (Db) and continuous reparative dentin extending along the lateral walls of dentin (arrows) and completely closing the exposure site. P: Pulp tissue (H&E x40). (b) A high magnification of Fig. (3a) showing layers of well-arranged odontoblasts (OL), a line of demarcation (blue arrow) between the primary dentin (D) and a thick layer of reparative dentin (yellow arrow). Notice the predentine layer (red arrow, H&E x200).

Figure 4

(a) A photomicrograph of group I (Biodentin group) after three months showing capping material on the exposure site (BD), a newly formed reparative dentine bridge (Db) completely closing the exposure site, cell inclusions inside the dentine bridge (yellow arrow), reparative dentine (RD) extending along the lateral wall of the pulp, line of demarcation (black arrows), blood vessels (red arrow) and pulp (P) tissue (H&E $\times 40$). (b) A high magnification of Fig. (4a) showing dental bridge (Db) with encapsulation of cells (yellow arrow) as well as mineralization exhibited more basophilic stain (black arrows) and pulp (P) tissue (H&E $\times 100$). (c) A photomicrograph of group I (BD group) after three months showing mature tall odontoblast layer (OL) along the lateral surface of dentine and abundant interlacing collagen (C) fibers (H&E $\times 200$).

Figure 5

(a) A photomicrograph of group II (Biosealer group) after one month showing capping material on the exposure site (BS), a thick almost complete reparative dentin bridge (Db) and tunnel defect (yellow arrow) near to the capping material. P: Pulp tissue (H&E $\times 40$). (b) A photomicrograph of group II (Biosealer group) after one month showing a tunnel defect near to the capping material (yellow arrow), vacuole-like spaces with various shapes sizes (arrow heads), complete degeneration of the pulp tissue, multiple

edematous spaces (*) and extravasated RBCs (red arrow, H&E $\times 100$). (c) A high magnification of Fig. (5a) showing a vacuolated odontoblastic layer along the lateral wall of the pulp (*), a layer of reparative dentin (yellow arrow) extending along the lateral wall of pulp (P) as well as a line of demarcation (red arrow) between primary dentin (D) and reparative dentine (H&E $\times 200$).

Figure 6

(a) A photomicrograph of group II (Biosealer group) after two months showing capping material on the exposure site (BS), an incomplete newly formed reparative dentin bridge (Db), with a tunnel defect (yellow arrow) and multiple calcified structures (bone or dentin-like structures) (blue arrows) scattered just beneath the dentin bridge as well as inside the pulp (P) tissue (H&E $\times 40$). (b) A high magnification of Fig. (6a) showing odontoblastic layer (OL) appeared as continuous layer and odontoblasts showing ill-defined boundaries. Notice the predentine (black arrow) and reparative dentine (yellow arrow) layers (H&E $\times 200$).

Figure 7

(a) A photomicrograph of group II (Biosealer group) after three months showing capping material on the exposure site (BS), a newly formed reparative dentine bridge (Db) completely closing the exposure site and pulp tissue (P) with a wide degenerated area (black arrow, H&E $\times 40$). (b) A photomicrograph of group II (BS group) after three months showing hyperemic vessels (black arrow), predentine layer (yellow arrow), reparative dentine along the lateral root canal wall (blue arrow), some new odontoblasts (red arrow) and calcified structures (H&E $\times 100$). (c) A photomicrograph of group II (BS group) after three months showing a new odontoblastic layer (OL), predentine layer (yellow arrow), reparative dentin present in the lateral walls (blue arrow) and almost a normal pulp (P) tissue (H&E $\times 200$).

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