Biological evaluation of a new pulp capping material developed from Portland cement

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\section*{A B S T R A C T}

This study evaluates the biological properties of a new pulp capping material developed from Portland cement. This study was conducted on 48 teeth in 4 dogs (12 teeth/dog). The dogs were classified into two equal groups (\textit{n} = 24 teeth) according to the evaluation period including: group A (3 weeks) and group B (3 months). Each group was further subdivided into three equal subgroups (\textit{n} = 8 teeth) according to the capping material including: subgroup 1: mineral trioxide aggregate (MTA), subgroup 2: Portland cement +\% 10 calcium hydroxide +\% 20 bismuth oxide (Port Cal) and subgroup 3: Portland cement + bismuth oxide. After general anesthesia, a class V buccal cavity was prepared coronal to the gingival margin. After pulp exposure and hemostasis, the capping materials and glass ionomer filling were placed on the exposure sites. All histopathological findings, inflammatory cell count and dentin bridge formation were recorded. Data were analyzed statistically. After 3 months, the histopathological picture of the pulp in subgroup 1 showed normal pulp, continuous odontoblastic layer and complete dentin bridge formation while subgroup 2 showed partial and complete dentin bridge over a normal and necrotic pulps. Subgroup 3 showed loss of normal architecture, areas of necrosis, complete, or incomplete dentin bridge formation, attached and detached pulp stones and fatty degeneration in group B. For group A, MTA subgroup showed the least number of inflammatory cell infiltrate followed by Port Cal subgroup. While subgroup 3 showed the highest number of inflammatory cell infiltrate. For group B, the mean inflammatory cell count increased with the three tested materials with no statistical difference. Regarding dentin bridge formation at group A, no significant differences was found between subgroups, while at group B, MTA subgroup exhibited significantly higher scores than other subgroups. In conclusion, addition of calcium hydroxide to Portland cement improves the dentin bridge formation qualitatively and quantitatively.

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1. Introduction

Pulp capping is defined as the treatment of exposed vital pulp by the application of capping materials to induce the dentinogenic potential of pulp cells (Schröder, 1985). The choice of pulp capping material greatly affects the success of vital pulp therapy. An ideal pulp capping material must be capable of inducing the formation of reparative dentin as well as acceptable biocompatibility and strong antibacterial activity (Mjör et al., 1991).

Calcium hydroxide is considered the gold standard of pulp capping materials. However, the resultant incomplete dentin bridge with tunnel defects that may lead to the failure of pulp capping (Faraco and Holland, 2001; Al-Hezaimi et al., 2011a). Mineral trioxide aggregate (MTA) was introduced by Torabinejad et al. (1993) and had been recommended as a pulp capping material. It has higher biocompatibility and sealing ability than calcium hydroxide (Parirokh and Torabinejad, 2010). Moreover, MTA can also induce the differentiation of dental pulp cells to odontoblast-like cells and form thicker dentin bridges (Masuda-Murakami et al., 2010; Al-Hezaimi et al., 2011a, b; Parirokh et al., 2011; Saleh et al., 2016). The success rate of direct pulp capping using MTA was found to be more successful than calcium hydroxide (Aguilar and Linsuwanont, 2011). However, MTA still has some limitations, including

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difficult handling characteristics, long setting time and relatively high cost.

The base material of MTA is Portland cement in which bismuth oxide has been added to render the mixture radio-opaque (Torabinejad et al., 1995a,b). Recently, the use of Portland cement as an alternative to MTA is gaining much popularity because of its lower cost and ample availability.

Several studies have been investigated the biocompatibility of Portland cement (Abdullah et al., 2002; Camilleri et al., 2005; Ribeiro et al., 2005; De Deus et al., 2005). These studies concluded that Portland cement is a biocompatible material having the potential to be used as a proper pulp-capping agent.

Our previous study showed that addition of 10 wt% calcium hydroxide to Portland cement associated with 20% bismuth oxide produces a new pulp capping material (Port Cal) with acceptable physical and adhesive properties (Negm et al., 2016). Therefore, the aim of the present study was to evaluate the biological properties of this new pulp capping material developed from Portland cement in dog’s teeth.

2. Materials and methods

2.1. Animals

This study was approved by the Ethics Committee at Faculty of Dentistry, Ain Shams University (2013/03END). A total of four male mongrel dogs aged approximately 4–6 months were selected for this study at the department of Surgery, Anesthesiology, and Radiology, Faculty of Veterinary Medicine, Cairo University. The dogs were bathed in Diazoin (Necoidal® Ningbo Hi-Tech Biochemicals, China) in concentration of 1/1000 ml of water and then were injected subcutaneously with Ivermectin (Ivomec® Merial Limited, Canada) at a dose of 200 μg/kg body weight for control of external and internal parasites. They were fed three days a time on cooked or dry food. Pure water was available all the time. All the dogs were monitored daily for any pathological conditions under supervision of an expert veterinarian.

Four teeth in three quadrants of each dog were included in the study summing up the total number of teeth to 48 (12 teeth/dog). The dogs were classified into two equal groups (n = 24 teeth) according to the evaluation period including: group A (3 weeks) and group B (3 months).

Each group was further subdivided into three equal subgroups (n = 8) according to the used capping material including: subgroup 1: MTA, subgroup 2: Portland cement + 10% calcium hydroxide + 20% bismuth oxide (Port Cal) and subgroup 3: Portland cement + bismuth oxide.

2.2. Formation of Port Cal

Bismuth oxide (LobaChemie, India) was incorporated into Portland cement (ASEC Helwan cement, Egypt) in the ratio of 20% by weight. The calcium hydroxide powder (ANALAR, Oxford laboratory, Mumbai, India) was then mixed with Portland cement in the ratio of 10% by weight.

The ingredients of the powder were blended together in a vibratory mixer for one hour. The resultant mixture was mixed with distilled water with a powder/water ratio 3:1 and the newly formed cement was designated Port Cal according to (Negm et al., 2016).

2.3. Procedure of pulp capping

The anesthetic regimen for each dog included subcutaneous injection of atropine sulphate (Atropine®, ADWIA, Egypt) at a dose of 0.05 mg/kg body weight and intravenous injection of xylazine HCl (Xylaject®, ADWIA, Egypt) 1 mg/kg body weight as a premedication. The anesthesia was induced by ketamine HCl (Ketamine®, EPICO, Egypt) 5 mg/kg body weight given i.v. via a 20 gauge cannula. The anesthesia was maintained during operation by 25 mg/kg incremental doses of 2.5% solution of thiopental sodium (Thiopental Sodium®, EPICO, Egypt) given i.v.

After general anesthesia, the teeth were disinfected by 0.5% povidone iodine solution (Betadine®, Nile company, Egypt). A class V buccal cavity was prepared approximately 1 mm coronal to the gingival margin with No. 2 Rose head carbide bur under copious normal saline irrigation in each tooth. Deepening of the pulpal floor for each cavity was done until the color of pulp tissue was reflected through the remaining dentin layer. Sterile sharp probe was used mechanically to expose the pulp. Bleeding was controlled by rinsing with sterile saline until the physiologic hemostasis occurred.

The capping materials were obtained by mixing the powder designated by each subgroup with distilled water on sterile glass slab using metal spatula to obtain a putty-like consistency; subgroup 1 “MTA” (Endocem Maruchi, Korea), subgroup 2 “Port Cal” and subgroup 3 “Portland cement + Bismuth oxide”. This mix was placed on the exposure sites by a fine amalgam carrier and condensed lightly with a moistened cotton pellet. Final restorations were done by insertion of glass ionomer filling (Riva, SDL, Australia). For pain and infection control, all dogs were given intramuscular cefotaxime sodium at a dose of 10 mg/kg and diclofenac sodium at a dose of 1.1 mg/kg once/day for 5 days after surgery (Abu-Seida, 2012)

2.4. Histologic examination

Dogs were sacrificed after each observation period by using 20 ml of 5% thiopental sodium solution rapidly injected through the cephalic vein. The maxilla and the mandible were removed surgically and sectioned into two halves at the midline. Blocks containing a single tooth with its surrounding bone were obtained by sectioning the jaws with a sharp saw. Burning of the other parts of the dead dogs was done in the medical waste incinerator at Faculty of Veterinary Medicine, Cairo University.

The teeth were fixed in 10% neutral buffered formalin for 72 h. Specimens were then decalcified in 17% EDTA solution for 120 days. A fine needle was used to perforate the specimens to allow EDTA penetration and the specimens were examined continuously for decalcification. After decalcification, specimens were dehydrated in ascending concentrations of ethanol then embedded in paraffin blocks. The embedded specimens were serially sectioned in buccolingual plane to the tooth main vertical axis, through the capping site and the pulp; into sections of 5 μm thickness. Serial sections that showed the deepest part of the cavity and the underlying pulp were selected for histological evaluation. These sections were stained with H&E for evaluation.

Histologic slides were examined under light microscope for the following assessments:

2.4.1. Inflammatory cell count: as described by (Tawfik et al., 2013)

For each slide, three representative fields were analyzed at ×200 magnification. Fields were selected conforming to the following criteria; i) well preserved tissue with good architecture and no artifacts, ii) intense inflammatory cells infiltration. Total inflammatory cell number was counted using image analysis software “Image J” software. The color coding threshold was adjusted to select the perimeter of the whole range of inflammatory cells in order to exclude other non-desired structures. Then binary thresholds of the selected color coded inflammatory cells were completed prior to calculation. The total number of cells was then counted as a factor of 10³.
2.4.2. Dentin bridge formation

Dentin bridge formation was graded by employing the scoring system reported by Min et al. (2009). Score 0: No dentin bridge, score 1: partial dentin bridge formation, and score 2: complete dentin bridge formation.

Dentin bridge thickness was assessed through the H&E stained sections using the image analysis software (Leica Queen 500).

Photomicrographs for H&E stained slides were captured by a digital camera (Leica, Wetzlar, Germany) attached to the light microscope. The magnification of the photos captured for analysis was fixed at (×40). The photomicrographs were transferred to a personal computer to be analyzed.

Through the set scale option of the image analysis software, the measurement unit (pixel) was converted into micrometer unit. The line measurement button in the tool bar was selected. By this tool ten lines were drawn perpendicular to the dentin bridge at the highest thickness to be measured. After that, by selecting 'analyze' “measure” the identified line was measured.

2.5. Statistical analysis

Data were analyzed using SPSS (Statistical Packages for the Social Sciences 22, IBM, Armonk, NY, USA). Quantitative data of inflammatory cell count were tested for statistical significance using ANOVA and multiple comparisons 'Duncan's test'. The results of scored data were tested with the Mann–Whitney U and Kruskal–Wallis non-parametric tests. Significance was established at P < 0.05.

3. Results

3.1. Histopathological findings

Histological examination of normal teeth revealed normal architecture of pulp consisting of delicate, loose connective tissue with numerous blood capillaries. A single uniform odontoblastic layer lining the peripheral part of the pulp and separating the loose connective tissue from predentin was seen. This layer is followed by cell rich zone with high densities of fibroblasts which were numerous, either spindle or stellate-shaped (Fig. 1).

3.1.1. Subgroup 1 (MTA)

After 3 weeks, the histopathologic examination of the superficial portion of the pulp showed destruction of odontoblastic layer opposite to the exposure site with loss of normal architecture of connective tissue and no dentin bridge formation. Areas of superficial necrosis could be noted. Deeper layers showed continuous odontoblastic layer and vasodilatation (Fig. 2a).

After 3 months, the histopathological examination of the superficial portion of the pulp tissue revealed regularity in its architecture in the area opposite to the exposure site with normal pulp, continuous odontoblastic layer and complete dentin bridge formation (Fig. 2b). The blood vessels were dilated and congested in superficial and middle portions of the pulp with few inflammatory cell infiltration.

3.1.2. Subgroup 2 (Port cal)

After 3 weeks, the histopathologic picture of the pulp showed continuous odontoblastic layer. No dentin bridge was formed at the exposure site with normal pulp (Fig. 3a). Dilatation and congestion of blood vessels and areas of necrosis were also evident in other sections (Fig. 3b).

After 3 months, the histopathological picture of the superficial portion of the pulp showed partial and complete dentin bridge over normal and necrotic pulps (Fig. 4), continuous odontoblastic layer, minimal pulp destruction and severe inflammation. The pulp also showed vasodilatation and localized necrotic areas. The pulp appeared to regain its integrity where few inflammatory cell infiltrates were observed in the middle of the pulp. Presence of attached and detached pulp stones were shown in some sections.

3.1.3. Subgroup 3 (Portland cement + Bismuth oxide)

After 3 weeks, the histopathologic picture showed continuous odontoblastic layer, vasodilatation, loss of normal architecture of connective tissue, and areas of necrosis. The deeper layers showed severe inflammation with inflammatory cell infiltration and no dentin bridge formation (Fig. 5).

After 3 months, the histopathologic picture showed continuous odontoblastic layer, vasodilatation, loss of normal architecture of connective tissue, and areas of necrosis. Some slides showed completely normal pulp while others showed complete or localized areas of necrosis. Areas of fibrosis was also evident. Deep layers showed inflammatory cell infiltration. Superficial layer at the exposure site showed complete, or incomplete dentin bridge formation as well as failed attempt to form dentin bridge (Fig. 6). Attached and detached pulp stones and fatty degeneration were also evident (Fig. 7).

3.2. Inflammatory cell count

The mean inflammatory cell count of different capping materials is shown in Table 1.

For group A (3 weeks), MTA subgroup showed the least number of inflammatory cell infiltrate followed by Port Cal subgroup. While Portland cement + bismuth oxide showed the highest number of inflammatory cell infiltrate. The difference in the mean inflammatory cell count between MTA and the other two materials was significantly high (P < 0.001).

For group B (3-months), the mean inflammatory cell count increased with the three tested materials at this period of time. However, MTA subgroup showed the least cell count, while Portland cement + bismuth oxide showed the highest inflammatory cell count with no significant difference.

3.3. Dentin bridge formation

Data were collected and tabulated in Table 2 and 3.

At group A (3 weeks), no significant differences was found between subgroups, while at group B (3 months), MTA subgroup exhibited significantly higher scores for dentin bridge formation than other subgroups.
Fig. 2. (a) HE stained histological section of a tooth of MTA subgroup after 3 weeks showing hard tissue deposition with vasodilatation, a big area of necrosis (N) and no dentin bridge formation. Magnification: 40x. (b) HE stained histological section of a tooth of MTA subgroup after 3 months showing complete dentin bridge formation (DB) with normal pulp and continuous odontoblastic layer (arrows) Magnification: 100x.

Fig. 3. (a) HE stained histological section of a tooth of Port Cal subgroup after 3 weeks showing the exposure site with normal pulp and no dentin bridge formation. Magnification: 100x. (b) HE stained histological section of a tooth of Port Cal subgroup after 3 weeks showing vasodilatation, areas of necrosis (N) and continuous odontoblastic layer (arrow) Magnification: 100x.

4. Discussion

The objective of the present study was to investigate the biological activity of newly developed material for pulp capping by mixing bismuth oxide and calcium hydroxide to the powder of Portland cement in comparison with MTA.

Mineral trioxide aggregate was developed to seal all of the pathways of communication between root canal system and external surface due to its good physicochemical properties and excellent biocompatibility (Lee et al., 1993; Hassanien et al., 2015).

In the present study 20% bismuth oxide, with 10% calcium hydroxide were added to Portland cement powder. The addition of bismuth oxide was done for its radiopacifying effect in order to simulate MTA (Bueno et al., 2009).

Calcium hydroxide was added in attempt to modify the biological characteristics of Portland cement. It has antimicrobial effect through destruction of bacterial cytoplasmic membrane, protein lysis and bacterial DNA damage. Also its mineralization effect which can be attributed to the hydroxyl ions released that induce an alkaline pH. This pH induces liquefaction necrosis in the superficial portion of the pulp, deeper portions of the pulp witness neutralization so stimulates hard tissue formation (El-Ashry et al., 2013; Revthi and Chandra, 2014).

In the absence of bacterial infection, a healthy pulp has a tremendous capacity to repair, a process clearly shown by the formation of a dentin bridge (Holland, 2008; Nagy et al., 2014).

In the present study, dog was the selected animal model due to the mechanism of induction and synthesis of dentin in this animal are the same as in human beings. Even though, the rate of reparative dentinogenesis may differ (Yamamura, 1985; El Ashry et al., 2016a). Dogs are believed to be a suitable experimental model because their pulp tissue is comparable to that of humans (El Ashry et al., 2016b).

Dogs dentition include four premolars and two or three molars in every quadrant which provides a good number of teeth allowing the comparison of more than one material in the same dog.

Pulp exposure was performed by mechanical perforation of the cavity floor with a sharp probe. This approach was recorded by several authors (Decup et al., 2000; Tsuneda et al., 1995) as it avoids extensive pulp damage caused by exposure during cutting with the bur and also creates a pulp exposure of uniform size.

In the present study, two evaluation periods were selected, the first period (3 weeks) in order to show the primary response of tissues and second period (3 months) for the final judgment of the procedure. Glass and Zander (1949) showed that not less than two weeks are needed for new odontoblasts to start to differentiate and form a barrier of new dentin.

In order to assess the biological capability of the tested materials, histopathological evaluation was included to determine the inflammatory reaction of pulp tissues and dentin bridge formation.

The inflammatory cell infiltrate differed with the different used materials and at different periods of time. During 3 weeks and 3
Fig. 4. (a) HE stained histological section of a tooth of Port Cal subgroup after 3 months showing the exposure site with partial dentin bridge formation (arrows). Magnification: 100×. (b) HE stained histological section of a tooth of Port Cal subgroup after 3 months showing inflamed pulp (IF) under complete dentin bridge (arrows). Magnification: 100×. (c) HE stained histological section of a tooth of Port Cal subgroup after 3 months showing normal pulp under complete dentin bridge (arrows). Magnification: 100×. (d) HE stained histological section of a tooth of Port Cal subgroup after 3 months showing normal pulp. Magnification: 100×.

Fig. 5. (a) HE stained histological section of a tooth of Portland cement with bismuth oxide subgroup after 3 weeks showing vasodilatation and continuous odontoblastic layer (arrows). Magnification: 100×. (b) HE stained histological section of a tooth of Portland cement with bismuth oxide subgroup after 3 weeks showing vasodilatation and necrosis (N). Magnification: 100×. (c) HE stained histological section of a tooth of Portland cement with bismuth oxide subgroup after 3 weeks showing severe inflammation. Magnification: 100×. (d) HE stained histological section of a tooth of Portland cement with bismuth oxide subgroup after 3 weeks showing no dentin bridge formation. Magnification: 100×.
Fig. 6. (a) HE stained histological section of a tooth of Portland cement with bismuth oxide subgroup after 3 months showing vasodilatation. Magnification: 100×. (b) HE stained histological section of a tooth of Portland cement with bismuth oxide subgroup after 3 months showing the exposure site with complete dentin bridge formation (DB) over necrotic pulp (N). Magnification: 100×. (c) HE stained histological section of a tooth of Portland cement with bismuth oxide subgroup after 3 months showing the exposure site with partial dentin bridge formation (arrows). Magnification: 100×. (d) HE stained histological section of a tooth of Portland cement with bismuth oxide subgroup after 3 months showing a failed attempt to form dentin bridge (arrows) over a necrotic pulp (N). Magnification: 100×.

Fig. 7. (a) HE stained histological section of a tooth of Portland cement with bismuth oxide subgroup after 3 months showing pulp stone (PS). Magnification: 100×. (b) HE stained histological section of a tooth of Portland cement with bismuth oxide subgroup after 3 months showing large detached pulp stone (PS). Magnification: 100×. (c) HE stained histological section of a tooth of Portland cement with bismuth oxide subgroup after 3 months showing fatty degeneration (FD). Magnification: 100×. (d) HE stained histological section of a tooth of Portland cement with bismuth oxide subgroup after 3 months showing pulp inflammation (IF) and necrosis (N). Magnification: 100×.
months periods MTA showed the least number of inflammatory cell infiltrate followed by Port Cal while Portland cement + bismuth oxide showed the highest number of inflammatory cell infiltrate. This finding could be due to difference in particle size, lack of quality control; in case of Portland cement; increased lead and arsenic content, lower calcium release and carbonation reaction accompanied with Portland cement (Camilleri, 2008).

Mineral trioxide aggregate is formed of a refined Portland cement with fine particles distribution that allows cells attachment and proliferation and promotes healing (Camilleri, 2008). This finding contradicts Hwang et al. (2009) who found similar inflammatory reactions between MTA and Portland cement. In this respect the statistical value of Port Cal was better than Portland cement + bismuth oxide which might be due to the higher calcium release provided by calcium hydroxide addition.

The thickness and homogeneity of the dentin bridge formed by MTA might be due to its fine and homogenous particles allowing tertiary dentin formation. This finding disagreed with Razmi et al. (2006) who found no statistical difference between dentin bridge formed by MTA and Portland cement as both have the same chemical composition.

As regards Port Cal, the presence of calcium hydroxide might have interfered with the hydration reaction of Portland cement giving rise to a weaker and porous structure that did not stimulate more hard tissue formation than other groups (Yoshida et al., 1995). This finding disagreed with Accorinte et al. (2003) who postulated that calcium hydroxide has a faster dentin bridge formation despite of inflammation. The reason for this contradiction could be related to the differences in the experimental subject where in case of Accorinte et al. (2003) study, human teeth were used. Also the amount of calcium hydroxide added in the present study was only 10% which might not be sufficient for hard tissue formation.

5. Conclusions

Under the conditions of the present study, it could be concluded that although MTA shows the least inflammatory response with the greatest percentage of complete dentin bridge formation yet, the addition of calcium hydroxide to Portland cement improves the possibility of dentin bridge formation qualitatively and quantitatively.

References


