INTRODUCTION

The material used for pulp capping affects the prognosis and success rate for the procedure. The desirable properties of a pulp capping material include good biocompatibility and hard tissue induction at the exposure site. Calcium hydroxide (CH) has been traditionally used for pulp capping due to its strong antimicrobial action and high alkalinity which induces dentin bridge formation. However, the limitations of CH include the resulting porous defects in the newly-formed dentin bridge, an inability to bond to dentin, and dissolution of the material in tissue fluid. Mineral trioxide aggregate (MTA) is a calcium-silicate (Ca-Si) based cement which has been successfully used in pulp capping. MTA is highly biocompatible, provides a bacterial seal, and can promote proliferation of human dental pulp cells with differentiation of odontoblasts. Moreover, MTA stimulated less pulpal inflammatory responses and more predictable reparative dentin formation compared with CH. Clinical studies also reported a higher long-term success rate when MTA was used as a pulp capping material, in comparison to CH. Nevertheless, one of the shortcomings of MTA is an extended setting time, with a potential risk of its seal being adversely affected during setting.

Recently, another Ca-Si based material, Bio-MA (M-Dent/SCG, Bangkok, Thailand), was introduced. Bio-MA and white mineral trioxide aggregate (WMTA) contain not only similar components such as tricalcium silicate, dicalcium silicate, and tricalcium aluminate, but also comparable physiochemical and biological properties. Bioactivity of Bio-MA, a calcium chloride accelerator-containing calcium-silicate cement, as a pulp capping material was evaluated on mechanically exposed rat molar pulp. Sixty maxillary first molars from Wistar rats were mechanically exposed and assigned to two capping materials: Bio-MA or white mineral trioxide aggregate (WMTA), and three periods: 1, 7, or 30 days. Nine molars were exposed and covered with polytetrafluoroethylene tape, as positive controls. From histological examination, inflammatory cell infiltration and reparative dentin formation were evaluated using grading scores. No significant difference in pulpal responses between the two materials was observed at any period (p>0.05). At 1 day, all experimental groups showed localized mild inflammation. At 7 days, dentin bridge was partially observed at exposure sites with few inflammatory cells. At 30 days, pulp appeared normal with complete tubular dentin bridges. Bio-MA with accelerator was biocompatible similar to WMTA and could be used as a pulp-capping material.

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Keywords: Biocompatibility, Calcium silicate cement, Dental pulp capping, Pulpal inflammation, Reparative dentin
Mechanical pulpal exposure and direct pulp capping

The rats were anesthetized by an intraperitoneal injection of 20 mg/kg Tiletamine hydrochloride and Zolazepam (Zoletil 50, Virbac, Bangkok, Thailand) and 5 mg/kg Xylazine hydrochloride (X-Lazine, MacroPhar, Bangkok, Thailand). Seventy-eight right and left maxillary first molars were used. Sixty molars in 30 rats were randomly allocated to 6 experimental groups, according to 1) pulp capping material of either Bio-MA or WMTA (Tables 1); and 2) one of three observation periods: 1, 7, or 30 days (n=10 per group). Using the contralateral split-mouth design, the left and right molars were randomly capped with Bio-MA or WMTA. Eighteen maxillary first molars were randomly chosen and used as controls. Nine teeth left intact served as negative controls (intact teeth, n=9). The remaining nine teeth as positive controls (uncapped pulp exposure, n=9) were divided into three follow-up periods (n=3 of each period).

Each tooth was cleaned and disinfected with a cotton pellet soaked in 2% chlorhexidine gluconate. Under magnifying loupes and a fiber-optic light (magnification 2.5×, Zeiss, Oberkochen, Germany), a sterile 0.8 mm slow-speed round diamond bur was used to prepare a cavity at mid-mesial surface, with copious irrigation of sterile normal saline, until the cavity depth was approximately half of the bur size. The bur was changed after every two cavity preparations in the same rat. Dental pulp was then exposed with a sterile sharp endodontic explorer (DG16, Dental USA, Mc Henry, IL, USA). Bleeding at the exposure site was controlled by pressing a sterile saline soaked cotton pellet over the exposure for 1–2 min. The perforation site was dried with paper points and directly capped with one of the pre-sterile capping materials. The pulp capping material was prepared according to the manufacturer’s instruction and carried onto the exposure site using the tip of the endodontic explorer. All cavities were restored with a light-cured glass ionomer cement (GC Fuji II LC, GC, Tokyo, Japan). For teeth acting as positive controls (uncapped), a sterile piece of polytetrafluoroethylene (PTFE, Teflon tape) was placed on the exposure site instead of the capping material, to prevent a direct contact between pulp and glass-ionomer cement restoration.

Table 1 Compositions of two pulp-capping materials used in this study

<table>
<thead>
<tr>
<th>Material</th>
<th>Lot no.</th>
<th>Compositions</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>White ProRoot MTA</td>
<td>0000144254</td>
<td>Calcium oxide, Silicon dioxide, Aluminum oxide, Bismuth oxide, Purified water</td>
<td>Dentsply Tulsa Dental Specialties, Tulsa, OK, USA</td>
</tr>
<tr>
<td>Bio-MA</td>
<td>01/2017</td>
<td>Calcium oxide, Silicon dioxide, Aluminum oxide, Bismuth oxide, Purified water, Calcium chloride</td>
<td>M-Dent/SCG, Bangkok, Thailand</td>
</tr>
</tbody>
</table>

Table 2 Evaluation criteria for rat pulpal response after direct pulp capping

<table>
<thead>
<tr>
<th>Score</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Inflammatory cell infiltration</td>
</tr>
<tr>
<td></td>
<td>None: absence or few scattering inflammatory cells</td>
</tr>
<tr>
<td>1</td>
<td>Mild: inflammatory cells limited to area of pulp exposure or dentin bridge</td>
</tr>
<tr>
<td>2</td>
<td>Moderate: inflammatory cells infiltration more than one-third, but not all of coronal pulp</td>
</tr>
<tr>
<td>3</td>
<td>Severe: all of coronal pulp is infiltrated with inflammatory cells or necrotic</td>
</tr>
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<table>
<thead>
<tr>
<th>Score</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Reparative dentin formation</td>
</tr>
<tr>
<td></td>
<td>None: no hard tissue deposition</td>
</tr>
<tr>
<td>1</td>
<td>Slight: scattered dentin bridge formation less than 50% of exposure site</td>
</tr>
<tr>
<td>2</td>
<td>Moderate: discontinuous dentin bridge formation is equal to or more than 50% of exposure site, but not completely covers the exposure site</td>
</tr>
<tr>
<td>3</td>
<td>Heavy: continuous dentin bridge completely covers the exposure site</td>
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<table>
<thead>
<tr>
<th>Score</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Presence of tubular structure (less than 50% of bridge area)</td>
</tr>
<tr>
<td>1</td>
<td>Presence of tubular structure (equal to or more than 50% of bridge area)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Score</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Absence</td>
</tr>
<tr>
<td>1</td>
<td>Presence of defective area or cell inclusion (less than 50% of bridge area)</td>
</tr>
<tr>
<td>2</td>
<td>Presence of defective area or cell inclusion (equal to or more than 50% of bridge area)</td>
</tr>
</tbody>
</table>
Histopathological procedure and evaluation

At 1, 7, and 30 days after direct pulp capping, the rats were euthanised through inhalation of carbon dioxide gas. The maxilla was sectioned and fixed in 10% neutral buffered formalin for 24 h at room temperature, then demineralized in 20% formic acid for 3 days. The maxilla was vertically separated into two halves, dehydrated through ascending concentrations of ethanol, and embedded into paraffin blocks. The 3-µm thick specimens were obtained by serial sectioning in the mesiodistal direction of maxilla. The specimens representing the area of entire pulp and the exposed area were selected and stained with hematoxylin and eosin (H&E staining, C.V. Laboratories, Bangkok, Thailand). The other specimens were stained with Modified Brown and Brenn (Bio-optica, Milano, Italy) to detect the absence or presence of bacteria in the capped pulp.

The stained sections were blindly evaluated by a trained investigator (T.P.) who was previously calibrated with an experienced pathologist (L.P.). Histopathological evaluation was performed twice with a two-week interval to confirm intra-observer reliability. Under a light microscope (Olympus BX53, Olympus, Tokyo, Japan) at the magnifications of 100×, 200×, and 400×, all samples were evaluated and scored for 1) inflammatory cell infiltration and 2) reparative dentin formation, according to the criteria modified from Liu et al.13) (Table 2). These scores between the two capping materials at three observational periods were compared using statistical analysis as stated below. The quality of reparative dentin formation was assessed in the 30-day specimens when dentin bridge was completely formed.

Statistical analysis

The data were statistically analyzed with the level of significance at p<0.05 by using IBM SPSS statistics software version 19 (IBM, Armonk, NY, USA). Inflammatory cell infiltration and reparative dentin formation were compared between the two capping materials using the Wilcoxon matched-pair signed rank test. The pulpal responses at the three observation periods were analyzed by Kruskal-Wallis test and pairwise comparison (Dunn’s test).

RESULTS

From the 78 rat molars initially included in this study, three teeth were excluded due to the dislodgement of the coronal restorations. The three teeth were one of each from the test groups: WMTA (7-day), Bio-MA (7-day), and a positive control (30-day). Inflammatory pulpal response and reparative dentin formation results are summarized in Fig. 1. From the Modified Brown and Brenn staining, all remaining specimens were free of
bacteria in the pulp capped areas.

**Inflammatory pulpal responses**

At 1, 7, and 30 days, all negative control groups showed intact odontoblastic layer and no pulpal inflammation (data not shown). At 1 day, mild to moderate inflammatory pulpal response (score 1–2) in the areas below the exposure were observed in the positive control group (Figs. 2A, D), WMTA (Figs. 2B, E), and Bio-MA (Figs. 2C, F) groups. Most of the specimens exhibited focal accumulation of polymorphonuclear leukocytes (PMNs), congested blood vessels, and local disruption of odontoblastic layer (Figs. 2D–F).

At 7 days, moderate to severe inflammatory response (score 2–3) was observed in the positive control group (Figs. 2G, J). From 7 out of 9 specimens in WMTA group (Figs. 2H, K) and 6 out of 9 specimens in Bio-MA group (Figs. 2I, L), no inflammation (score 0) with an absence or scattering of inflammatory cells were observed at the exposure areas. A mild inflammatory response (score 1) was observed in the remaining specimens capped with WMTA (two) and Bio-MA (three) respectively.

At 30 days, severe inflammatory responses (score 3) were observed in the positive control group (Figs. 3A, D). Nine out of 10 specimens in either WMTA (Figs. 3B, E) or Bio-MA (Figs. 3C, F) group exhibited no inflammation.
Fig. 3  Histological sections stained with H&E at 30 days. D–F present a high-magnification view (200×) of the areas demarcated by the black rectangulars in A–C (100×). G–L present a high-magnification view (400×) of the areas demarcated by the black dotted rectangulars in A–C. (A, D, and G) Severe inflammation with moderate hard tissue deposition at exposure area in positive control group (PTFE). Pulp capped with WMTA (B, E, and H) and Bio-MA (C, F, and I) appeared normal with re-organization of pulpal cells and no inflammation. Tubular dentin bridge with minimal defective areas was completely formed at exposure area. Note the well-organized layer of polarizing odontoblastic cells indicated by the black arrows. * Pulp exposure site; P, pulp; D, dentin; RD, reparative dentin.

(score 0). WMTA- and Bio-MA-capped pulp appeared normal with re-organization of pulpal cells and odontoblast-like cells under the newly formed dentin bridge. Only one specimen in each group presented a mild inflammatory response (score 1).

No significant difference in the inflammatory responses was observed between the two capping materials at any observation period (p>0.05). For each capping material, significant difference in the inflammatory responses was observed between 1 and 30 days (p<0.05, Fig. 1A).

Reparative dentin formation
At 1 day, no hard tissue barrier was formed in any specimen of the positive control, WMTA and Bio-MA groups (score 0, Figs. 2A–F).

At 7 days, focal calcification was observed at a distance below the exposure area in the positive control group (score 0, Figs. 2G, J). While a layer of newly-generated mineralized matrix was detected subjacent to WMTA and Bio-MA. Nearly half of the specimens in WMTA (Figs. 2H, K) and Bio-MA (Figs. 2I, L) groups showed moderate hard tissue deposition (score 2), and the other half showed heavy hard tissue deposition (score 3).

At 30 days, all specimens in the positive control group showed moderate hard tissue deposition (score 2) with non-tubular structure (score 0) and large cell inclusion areas (score 2) (Figs. 3A, D, and G). The majority of specimens capped with WMTA (Figs. 3B, E, and H) and Bio-MA (Figs. 3C, F, and I) exhibited heavy hard tissue deposition completely covering the exposure sites (score 3) with tubular structure≥50% (score 2) and cell inclusion with area<50% of dentin bridge.

Formation of hard tissue barriers at 7 and 30 days in WMTA and Bio-MA groups was significantly higher than that at 1 day (p<0.01). No significant difference existed in quantity or quality of reparative dentin formation between the two capping materials (p>0.05) (Figs. 1B, C).

DISCUSSION
This study confirmed that direct pulp capping with both
Bio-MA, the calcium chloride-containing Ca-Si based cement and WMTA induced complete pulpal repair in mechanically exposed rat molars within 4 weeks.

Pulpal responses at 1, 7, and 30 days after direct pulp capping with the Ca-Si cements were consistent with previous studies. At 1 day, mild acute inflammatory response was observed in all specimens and most likely contributed by the mechanical injury of the simulated pulpal exposure. Inflammation in the pulp capped with Bio-MA and WMTA subsided at 7 days with the distinctive signs of pulp repair indicated by a partial or complete formation of reparative dentin bridge at the exposure sites. At 30 days, the dentin bridge was completely mineralized with thick, homogeneous and tubular structures, and alinged with a well-organized odontoblastic layer. However, pulp healing in human teeth is commonly much slower and may take up to 3 months to obtain such results.

Pulp capping with either Bio-MA or WMTA showed no significant difference in inflammatory reactions and reparative dentin formation. This could be expected due to the similarity in their main ingredients including tricalcium silicate, dicalcium silicate, and tricalcium aluminate (M-Dent/SCG internal data). The exception is the addition of CaCl2 in the liquid component of Bio-MA as an accelerator during setting. From the results of our study, CaCl2 did not negatively or positively affect the pulp healing after direct pulp capping.

The quantity and quality of dentin bridge formed in the pulp capped with WMTA and Bio-MA were considerably higher than that in the uncapped pulp covered with PTFE at 7 or 30 days. For the capped pulp, Ca-Si based cements were moistened with tissue fluid from pulp region. Tri- and di-calcium silicate became hydrated to form calcium silicate hydrate gel and CH. Calcium (Ca) ions releasing from the hydrated cement had beneficial effects on pulpo-dentin complex. Firstly, the regeneration and differentiation of odontoblast-like cells were promoted by the release of Ca ions from MTA. The odontoblast-like cells replaced the damaged odontoblasts and formed the mineralized tissue at the exposure sites. Secondly, the Ca ions directly reacted with phosphate ions in tissue fluid to form hydroxyapatite crystals, which were incorporated into the reparative dentin bridge.

Auto-deposition of reparative dentin matrix even in the absence of the capping material has been previously reported in studies using rat molars. To distinguish the inducing effect of capping materials from the self-repairing reaction, PTFE, a chemically inert material was suggested to be used in uncapped pulp as a positive control. PTFE tape prevented a direct contact between pulp tissue and the restorative material, allowing an evaluation of rats’ own pulp responses. In our study, pulp covered with PTFE tape showed persistent moderate chronic inflammation with the partially formed, mineralized tissue in osteoid-like and atubular structure, similarly to the results of the previous studies. The slow resolution of inflammation and dentin bridge formation in the uncapped pulp confirmed the self-repair capacity of rat molar pulp and the potential in regenerating and differentiating of pulpal cells of the Ca-Si based capping materials.

CONCLUSION

The two Ca-Si based cements, with or without calcium chloride accelerator, indistinguishably induced pulp healing and reparative dentin formation of mechanically exposed pulp in rats. With a reduced setting time, Bio-MA might provide a clinical advantage and can be used as a promising pulp-capping material, similar to WMTA.

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REFERENCES

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