Evaluation of Pulp Response to Novel Bioactive **Glass Pulp Capping Materials**

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Abstract

Introduction: This study aimed to investigate dental pulp responses to novel bioactive glass (BG) pulp capping materials after direct pulp capping in vivo. Methods: Novel BG pulp capping materials are composed of powder and fluid. The powder is BG (82.36% SiO₂, 15.36% CaO, and 2.28% P₂O₅), and the fluid is provided in 2 kinds: (1) phosphate buffer solution (BG-PB) and (2) phosphate buffer solution with the addition of 1 wt% sodium alginate (BG-PB-SA). After mixing the powder and fluid, BG-PB and BG-PB-SA were prepared. Cavities with mechanical pulp exposure were prepared on maxillary first molars of Wistar rats. The exposures were randomly capped with BG-PB, BG-PB-SA, or mineral trioxide aggregate (MTA). After 1 (n = 6) and 4 weeks (n = 8), maxillary segments were obtained and prepared for histologic analysis with a scoring system. Statistical analysis was performed using the Kruskal-Wallis and Mann-Whitney U tests with the significance set at .05. Results: After 1 week, few inflammatory cells were present in the BG-PB, BG-PB-SA, and MTA groups. Moreover, a thin layer of newly generated matrix was observed in most specimens. After 4 weeks, all specimens from the 3 groups formed a heavy dentin bridge. BG-PB and BG-PB-SA groups exhibited no or slight inflammatory response, whereas the MTA group exhibited a slight to moderate inflammatory response. No significant difference was observed in pulp inflammation and dentin formation among the 3 groups at either time point (*P* > .05). **Conclusions:** When used as a pulp capping agent, BG-PB and BG-PB-SA had similar favorable cellular and inflammatory pulp responses to those of MTA. Therefore, BG is a promising pulp capping material. (J Endod 2017; ■:1-4)

Key Words

Bioactive glass pulp capping material, direct pulp capping, mineral trioxide aggregate

Dulp capping materials are important in the success rate of direct pulp capping (1-3). An ideal pulp capping material should exhibit good sealing ability, good biocompatibility, strong antibacterial activity, and easy handling.

Significance

A pulp capping agent can influence the success rate of direct pulp capping. We proposed promising bioactive glass pulp capping materials that showed similar favorable results with MTA when capped in a rat model.

Calcium hydroxide (CH) is a conventional pulp capping material. However, it is a bioinert material, and it forms an incomplete dentin bridge with tunnel defects (4, 5). Mineral trioxide aggregate (MTA) is a bioactive material that has been recently applied in direct pulp capping. More homogenous (fewer tunnel defects) dentin bridges would form with MTA capping than with CH (4). MTA also obtained a higher clinical success rate than CH (1-3). Nevertheless, MTA still presents limitations, such as a long setting time, difficult handling characteristics, and cytotoxicity during the initial setting phase (6). Recently, several calcium silicate-based bioceramic materials, such as iRoot BP Plus (Innovative Bioceramix, Vancouver, Canada) and Biodentin (Septodont, St Maur des Fossés, France), have been reported to exert similar effects with MTA in pulp capping (4, 7-9), but clinical studies are lacking.

Bioactive glass (BG) is mainly composed of CaO, SiO₂, and Na₂O. Given that BG possesses a noncrystalline structure, it should exhibit better bioactivity than that of other bioceramics with a crystalline structure, such as MTA and iRoot BP Plus. BG presents good antibacterial properties (10) and induces proliferation, differentiation, and mineralization of human dental pulp cells (11, 12). Moreover, BG induces dentinlike tissue formation when transplanted with pulp tissue in the dorsum of nude mice (12). On the basis of the excellent BG properties, novel BG pulp capping materials were synthesized. The present study aimed to investigate pulp inflammation and dentin formation after direct pulp capping with novel BG pulp capping materials in vivo.

Materials and Methods

Preparation of Materials

BG pulp capping materials are composed of powder and fluid. The powder is BG (82.36% SiO₂, 15.36% CaO, and 2.28% P₂O₅) synthesized by using the sol-gel method combined with template technology. Two kinds of fluid are provided:

- 1. Only phosphate buffer solution (BG-PB)
- 2. Phosphate buffer solution with the addition of 1 wt% sodium alginate (BG-PB-SA)

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BG-PB and BG-PB-SA were obtained after mixing powder and fluid on a glass slab at a powder-to-liquid ratio of $1.4 \sim 1.5$ g/mL. BG pulp capping materials are easy to handle. The initial and final setting times were 5 to 7 minutes and 9 to 12 minutes, respectively. These materials were synthesized by the National Engineering Research Center for Tissue Restoration and Reconstruction of South China University of Technology, Guangzhou, China. ProRoot MTA (Dentsply Tulsa Dental, Tulsa, OK) was mixed with distilled water in accordance with the manufacturer's instructions.

Animals

This study was approved by the Ethics Commission of Peking University Health Science Center, Beijing, China. A total of 46 maxillary first molars from 23 male Wistar rats (180–200 g) were used for this *in vivo* study. Two time points were designed for observing pulp responses. Four teeth were used as negative controls, and 2 were used for each time point. Eighteen and 24 teeth were used for 1 week and 4 weeks, respectively. Each left and right first maxillary molar was capped using different materials.

Direct Pulp Capping Procedure

After being anesthetized with an intraperitoneal injection of 2% pentobarbital, the rats' teeth were cleaned and disinfected with cotton soaked in 75% ethanol. With the aid of a dental microscope (10.4-17.0× magnification) (OMS2350; Zumax Medical Co, Ltd, Suzhou, China), cavities were prepared on the mesial surfaces of the maxillary first molar using a sterilized ultrasonic tip (CAP 3; Satelec, Merignac, France). To avoid pulp impairment from heat, the teeth and cutting instruments were irrigated with sterile distilled water. Pulp was exposed using the tip of a #15 sterile stainless steel file through the remaining thin dentin of each cavity. Bleeding was controlled by pressing sterile paper points for a few seconds. The pulp exposure sites in the 3 experimental groups were directly capped with BG-PB, BG-PB-SA, or MTA. In the negative control group, the pulp was not capped with any pulp capping agent. All cavities were subsequently restored with glass ionomer cement (Fuji IX; GC International Corp, Tokyo, Japan) according to the manufacturer's instructions. The cusp tips of the opposing teeth were cut to minimize occlusal forces.

Sample Preparation and Histologic Analysis

The animals were anesthetized and sacrificed at 1 and 4 weeks after surgery. The maxillary sections, together with molars, were dissected and fixed in 4% paraformaldehyde for 24 hours at 4°C. The tissues were demineralized in 10% EDTA/phosphate-buffered saline solution and embedded in paraffin. The 5 μ m-thick serial sections were cut in the mesiodistal direction. The slices were numbered, and the median sections were selected for hematoxylin-eosin staining.

Two observers were trained to evaluate the histologic features according to the criteria presented in Table 1 (9, 13, 14). The criteria of quality of dentin formation in the bridge were used to assess those of

4-week specimens only. Formal assessments were started when the 2 observers obtained 95% consistency. All specimens were rearranged and numbered. When disagreement existed between the 2 observers, they discussed it until a consensus was reached.

Statistical Analysis

The histologic evaluation results were analyzed statistically using the Kruskal-Wallis and Mann-Whitney U tests, with the level of significance set at .05.

Results

Pulp Inflammatory Response

At week 1, a moderate inflammatory response was observed in the negative control group (Fig. 1*A* and *E*). The BG-PB, BG-PB-SA, and MTA groups showed similar inflammatory cell responses (Table 2). Most of the specimens in the 3 groups exhibited no or few inflammatory cells (Fig. 1*B*–*D* and *F*–*H*). The remaining specimens showed mild inflammatory responses. No significant difference in the inflammatory responses was observed among the 3 groups (P > .05).

At week 4, necrosis was observed in the negative control group (Fig. 2*A* and *E*). All specimens in the BG-PB group exhibited no or few inflammatory cells (Fig. 2*B*, *F*, and *I*). In the BG-PB-SA group, 2 specimens showed mild inflammatory responses, and 6 specimens showed no or few inflammatory cells (Fig. 2*C*, *G*, and *J*). In the MTA group, 2 specimens exhibited moderate inflammation, 2 specimens showed mild inflammatory responses, and 4 specimens showed no inflammatory responses (Fig. 2*D*, *H*, and *K*). No significant difference in the inflammatory responses was observed among the 3 groups (P > .05).

Hard Tissue Formation

At week 1, no hard tissue formed in the negative control group (Fig. 1*A* and *E*). In the BG-PB-SA and MTA groups, a slight layer of newly generated matrix next to the material was observed in 5 of 6 specimens (Fig. 1*C*, *D*, *G*, and *H*). Only half of the BG-PB group specimens exhibited mild hard tissue deposition (Fig. 1*B* and *F*). No significant difference was observed in hard tissue formation among the 3 groups (P > .05).

At week 4, an incomplete dentin bridge was observed in the negative control group (Fig. 2*A* and *E*). All specimens in the 3 experimental groups showed heavy hard tissue formation. Nearly all specimens presented irregular or regular tubular patterns in the newly formed calcified bridge except for 2 MTA group samples (no tubules present). The BG-PB, BG-PB-SA, and MTA groups formed well-organized tubular dentin bridges in 6, 4, and 2 samples, respectively (Fig. 2*B*–*D* and *F*–*H*). No significant difference existed in dentin bridge quality among the 3 groups (P > .05). The evaluation scores of each group after direct pulp capping are presented in Table 2.

TABLE 1. Criteria Used for the Histologic Analysis of the Pulps Treated with Direct Pulp Capping

Grade	Hard tissue formation	Inflammatory cell response	Quality of dentin formation in the bridge
1	Heavy: hard tissue deposition as complete and continuous dentin bridge	Absent or few inflammatory cells	Regular pattern of tubules
2	Moderate: hard tissue formation as incomplete and discontinuous dentin bridge	Mild: inflammatory cells only next to dentin bridge or area of pulp exposition	Irregular pattern of tubules
3	Slight: a layer of scattered and foggy hard tissue deposition	Moderate: inflammatory cells are observed in the part of coronal pulp	No tubules present
4	No hard tissue deposition	Severe: all coronal pulp	—

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