Original

Experimental Study of Pulp Capping Using Xenogenic Demineralized Dentin Paste

Ji-Young Yun1, Yong-Hoon Choi1, Young-Kyun Kim2,3,4, In-Woong Um6, Joo-Cheol Park3,4,5 and Ji-Yoon Kim7

1) Department of Conservative Dentistry, Section of Dentistry, Seoul National University Bundang Hospital, Seongnam, Korea
2) Department of Oral and Maxillofacial Surgery, Section of Dentistry, Seoul National University Bundang Hospital, Seongnam, Korea
3) Department of Dentistry, School of Dentistry, Seoul National University, Seoul, Korea
4) Dental Research Institute, School of Dentistry, Seoul National University, Seoul, Korea
5) Department of Oral Histology, School of Dentistry, Seoul National University, Seoul, Korea
6) R&D Institute, Korea Tooth Bank, Seoul, Korea
7) Department of Science Education, College of Education, Dankook University, Yongin, Korea

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Abstract: This study aimed to compare the effectiveness of demineralized dentin paste (DDP) with mineral trioxide aggregates (MTA) as a direct pulp capping material. Artificial Class V cavity was prepared to make a pinpoint pulp exposure in a third of the buccal cervical region of maxillary and mandibular right/left canine teeth of six beagle dogs. After bleeding control, (hemostasis was confirmed), MTA (n= 12) was applied to the maxillary and mandibular right canines. DDP (n=12) to the left canines as a control. Superior to the pulp-capped area, the inferior 1/3 of the cavity was filled with resin modified glass ionomer, and the rest of the cavity was filled with a microfilled composite resin using one-step self etching adhesive system. Two, four, and eight weeks later, the dogs were sacrificed and the capped portion was sectioned perpendicularly to the tooth longitudinal axis of tooth to get specimens for microscopic examination. MTA formed a calcific bridge of osteo-dentin in the histological specimens from the dogs sacrificed two, four, and eight weeks later. DDP formed no calcific bridge in the histological specimens from the dogs sacrificed two weeks later but formed a physiologic dentin bridge close to actual dentin, which included dentinal tubules, in those from the dogs sacrificed four, eight weeks later. DDP-using pulp capping led to formation of a physiological dentin bridge and DDP is expected to be applicable as an effective, biocompatible material for direct pulp capping if sealing ability and easiness for handling materials are improved through further research on its properties.

Key words: Pulp capping, Demineralized dentin matrix, MTA

Introduction

It is important to keep pulp vitality at the maximum in treating teeth damaged by dental caries, injuries, and others. Pulp plays a crucial role in forming dentin during the process of tooth development (odontogenesis)1,2. It may contribute to formation of reactive, reparative, and sclerotic dentin in case of pathological stimulation even after odontogenesis3. It is known that such inherent ability allows exposed pulp to be cured through cell reorganization and bridge formation if microleakage from the outside can be prevented effectively3. On the basis of this knowledge, direct pulp capping using various materials has long been performed4-6. It has traditionally been known that pulp capping using calcium hydroxide may lead to formation of a dentin bridge as calcium ion induces cell migration, proliferation, and differentiation and hard tissue deposition7. In contrast, there were reported that tunnel defects present in this dentin bridge could become a passage for microleakage and cause inflammatory transformation of pulp and their report is highly controversial8-10. Some researchers report that using Mineral trioxide aggregates (MTA) composed of tricalcium silicate, dicalcium silicate, tricalcium aluminate, calcium sulfate and bismuth oxide for pulp capping can be quicker, form a thicker dentin bridge, and be less likely to cause inflammatory reaction, pulp hyperemia, and necrosis than using calcium hydroxide11-13. It is known that MTA, which includes no calcium hydroxide, forms calcium oxide when it hardens and reacts to tissue fluid and forms calcium hydroxide14. Koh et al.15 reported that MTA stimulated osteoblasts to secrete cytokine and that the MTA calcium phosphate phase induced osteoblast adhesion. However, MTA may be at risk of microleakage

Correspondense to: Dr. Young-Kyun Kim, Department of Oral and Maxillofacial Surgery, Section of Dentistry, Seoul National University Bundang Hospital, 300 Gumi-dong, Bundang-gu, Seongnam city, Gyeonggi-do, Korea; Tel: +82-31-787-2780; Fax: +82-31-787-4068; E-mail: kyk0505@snubh.org & kyk0505@daum.net

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in case of initial setting since it takes more than two hours to set, induce tooth discoloration, be expensive, and be hard to manipulate. Ideal materials for successful direct pulp capping should basically be biocompatible, form a dentin bridge to provide good sealing, and prevent infiltration of bacteria to minimize pulpal inflammatory reaction. In the past a small piece of gold had been used on direct pulp capping. It has been reported that demineralized dentin matrix (DDM) made from properly processed teeth has almost the same composition as bones and may induce osteogenesis if it is grafted in an intraosseous edentulous area or even in soft tissues, such as muscles. DDM is also known to cause differentiation of dental pulp stem cells (DPSCs) into odontoblast-like cells and induce secondary dentinogenesis when it touches pulp cells.

This study aimed to compare pulpal reaction between DDM and MTA by applying both of them to the exposed pulp and assess capability and values of DDM as a material for pulp capping.

Materials and Methods

Fabrication of DDP

Extracted bovine teeth were refrigerated in ethyl alcohol. In the Korea Tooth Bank (Seoul, Korea), they were washed to remove foreign matters—prosthesis, restoration, calculus, etc.—and soft tissues (Korea Patent Number 10-1062381), separate the crown from the root, and remove pulp completely from the root, which was then washed with each of distilled water, oxygenated water, and an ultrasonic cleaner for twenty minutes by stages. A grinder was used to grind the root into 80-400 µm grains of powder, which was then soaked into a 99 % chloroform methanol solution to defat, centrifuged at 4,000 rpm for three minutes to remove floating fat, and washed with distilled water. Then, it was demineralized through three-minute shaker stirring using 0.5 N HCl at a temperature of 18-20°C and was dehydrated and defatted through thirty-minute shaker stirring using ethyl alcohol and methanol solutions. It was washed for ten minutes with distilled water three times; then, it was freeze-dried for 3-5 hours. The demineralized dentin powder was kept at a room temperature out of the direct rays of the sun.

A grinder was used to make 10-20 µm grains of fine powder. Then, the fine powder was put into a plastic container for centrifugation, mixed it with distilled water in a volume ratio of 1:1, and run a centrifuge (OrthoMTAautomixer, BioMTA, Seoul, Korea) for 15 seconds to transform it into a form of paste. A cotton swab was used to remove remaining water and used a paste carrier (BioMTA, Seoul, Korea) to apply the paste to required areas (Fig. 1).

Animal experiment

This animal experiment was conducted with the approval of the Institutional Animal Care and Use Committee (IACUC), Seoul National University Bundang Hospital (BA1409-160/047-01). It complied with the ARRIVE guidelines. 10 mg Ketamine HCL (Zoletil 50, Virbac, Carros, France) and 0.2 mg Xylazine HCL (Rumpen, Bayer Korea, Seoul, Korea) was injected intravenously to six 12-month-old beagle dogs to put them under general anesthesia. 2 % lidocaine containing 1:100,000 epinephrine (Lidocaine HCL Injs, Yuhan Corps., Seoul, Korea) was used to give infiltration anesthesia around maxillary and mandibular right/ left canines. Then, class V cavity (2 mm in diameter and 1.5 mm in depth) was made in a third of the buccal cervical region of the canine teeth and intentionally made a pinpoint pulp exposure. (used a pinpoint to expose the pulp intentionally.) A saline solution was used to wash the exposed area and put sterile cotton pellet on the area for 60 seconds to control bleeding (hemorrhage); After bleeding was controlled, (when hemostasis was confirmed,) mineral trioxide aggregates (ProRoot™ MTA, Dentsply, Tulsa Dental, Tulsa, Oklahoma, USA) and DDP were applied to pulp exposed area for capping. MTA was applied to the maxillary and mandibular right/ left canines. Resin modified glass ionomer (RMGI) (GC Fuji II LC, GC Co., Tokyo, Japan) was applied to cover the pulp capped area, and the bonding agent OptiBond All-In-One (Kerr Corp.,
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Figure 3. 2nd week histologic findings (H-E stain, scale bars = 200 μm).
A: MTA group. A calcified bridge (CB) is well formed to cover the entire opening along the tooth injury area. Odontoblasts are observed together with predentin (white arrows) along the existing dentin.
B: High magnification of black box of Fig.3A. Odontoblast-like cells (black arrows) are arranged under the calcified bridge. The new calcified bridge (CB) of osteo-dentin is showing irregular features with entrapment of cells resembling osteocytes.
C: DDP group. There is no inflammatory finding. No dentin bridge is formed along the tooth injury area.
D: High magnification of black box of Fig. 3C. No hard tissue formation is observed around tooth powder.

Orange, USA) was used to restore the cavity with the filling material, flowable resin (3M ESPE Dental Products, St. Paul, Minnesota, USA) after pulp treatments. It is known that the biocompatibility of RMGI is better than composite resin materials because an acidic monomer of an adhesive bonding system would irritate the pulp21). However, the mechanical properties of the composite resin are better than RMGI. Therefore, a sandwich technique in which conventional and resin modified glass ionomers are used as bases under resin composite has been recommended to satisfy both the biocompatibility and the mechanical strength22). Therefore, in this study, 0.5mm (1/3 depth of the cavity) RMGI base was applied superior to the pulp-capped area, and then the rest of the cavity was filled with a composite resin, which has better mechanical properties than RMGI (Fig. 2).

**Animal sacrifice and histological observation**

The animals were sacrificed two, four, and eight weeks later. Feeding was prevented for twelve hours before sacrifice the animals, then injected 0.005 mg/kg atropine sulfate (Atropine, DAI HAN Pharm. Co. Hwasung, Korea) as an anesthetic hypodermically, and, about 15 minutes later, injected 0.2 mg/kg xylazine (Rompun, Bayer, Seoul, Korea) and 5 mg/kg zoletil (Zoletil 50, Virbac SA, Seoul, Korea) intramuscularly for general anesthesia. External jugular vein cannulation and common carotid artery cannulation was performed to injection of 5ml KCl (potassium chloride, JW Pharmaceutical) for sacrifice. A stethoscope was used to confirm cardiac arrest and injected 10% formaldehyde into common carotid artery to give perfusion fixation of teeth in the experimental area. The block of teeth along with neighboring alveolar bone in the experimental area was collected from each dog, then were fixed immediately in 10% neutral buffered formalin. The tissues were washed and dehydrated in 80-100% ethanol, and then embedded in methacrylate-based chemical curing resin. Sample blocks were sliced perpendicularly to tooth longitudinal axis and were grinded by 40-50 μm grains. Optical microscope (BX51, Olympus, Tokyo, Japan) observation was performed after H-E staining.
Results

2nd week histologic finding

**MTA group**: There was no any specific finding such as inflammation. Calcified bridge was well formed to cover the entire opening along the tooth injury area in MTA group tissues. Odontoblasts were observed together with predentin along the existing dentin (Fig. 3A). In contrast, odontoblast-like cells were arranged under the calcified bridge. The new calcified bridge was osteo-dentin showing irregular features with entrapment of cells resembling osteocytes (Fig. 3B).

**DDP group**: There was no inflammatory finding in DDP group tissues. Dentin bridge was not formed along the pulp-capped area with DDP. No any hard tissue formation was observed around tooth powder (Fig. 3C, 3D).

4th week histologic finding

**MTA group**: A thick calcified bridge of osteo-dentin with cells was formed below the pulp-capped area (Fig. 4A, 4B).

**DDP group**: There was no inflammatory finding; a dentin bridge with well-developed dentinal tubules (dentine tubules in the form of slant lines were observed) was well formed below the pulp-capped area; and hard tissue formation was observed around tooth powder (Fig. 4C, 5D).

8th week histologic finding

**MTA group**: A thick osteo-dentin bridge covered the pulp-capped area completely without dentinal tubule observed. There were few odontoblast-like cells under the bridge (Fig. 5A, 5B).

**DDP group**: A dentin bridge composed of reparative dentin with irregular dentinal tubules and underlining odontoblast-like cells was well formed along the pulp-capped area (Fig. 5C). In another specimen, there was a hard tissue bridge composed of tooth powder and surrounding newly formed mineralized matrix in the exposed pulp (Fig. 5D).

Discussion

Direct pulp capping is known to be clinically effective therapy for permanent teeth and its success rate is considered to range from 80% to 90% \(^{23}\). Successful pulp capping requires a high level of biocompatibility of materials, a low level of cytotoxicity, and good sealing as well as selection of proper cases. Recently, lots of
studies mainly using MTA and calcium hydroxide were conducted; as a result, no statistical difference in the success rate was observed between them but indirect comparison showed that MTA was better\(^2\). An experimental study found that pulp capping using hyaluronic acid was effective in forming reparative dentin\(^2\). Bae et al. conducted an experimental study on pulp capping using diverse materials\(^2\). They reported that calcium hydroxide and photopolymerized calcium hydroxide were proper as direct pulp capping materials because odontoblasts and a dentin bridge were formed following initial pulp inflammatory reaction, whereas adhesive resin caused severe inflammatory reaction and pulp necrosis.

Pulp capping may be successful when external stimuli and micro-leakage are blocked by applying biocompatible capping materials and when a hard-tissue bridge is formed by controlling inflammation\(^2\). Many researchers have reported that MTA is an effective pulp capping material in forming a dentin bridge and research on its mechanism is actively being conducted\(^2\). MTA promotes proliferation of the human DPSCs, and DPSCs secrete VEGF and FGF-2, which are imperative for cell migration and inflammation repair\(^3\). In forming dentin, odontoblasts secrete noncollagenous proteins; of these, dentin sialoprotein (DSP) and alkaline phosphatase (ALP) are known to play a crucial role in mineralization of reparative dentin\(^3\). The fact that DPSCs cultivated in MTA showed a high level of DSP and ALP expression can mean that DPSCs were differentiated into odontoblast-like cells\(^3\). MTA induces cytokine secretion for osteoblast adhesion; pulp cells cultivated in MTA are known to be differentiated in a similar way to those cultivated in BMP-4 and such a property may reportedly contribute to formation of a calcific barrier\(^4\). Such an effect of MTA was also observed in the histological finding in this study: osteo-dentin widely formed around the MTA-capped area. A homogenously thick, good-quality calcific bridge is formed; however, it is mainly composed of osteo-dentin and is histologically different from actual dentin.

Noncollagenous proteins within demineralized dentin are known to facilitate odontoblast differentiation or angiogenesis\(^5\). It has also been reported that DDM acts as a scaffold for
undifferentiated mesenchymal cells to make mitogenic and chemotactic activity. Another report is that DDM has the capability to form periapical hard tissues at a similar level to calcium hydroxide and can be used as an apexifying agent. On the basis of the contribution of DDM to hard tissue formation, we used DDM to develop a pulp capping material and applied the material to the pulp exposure area to assess its histological changes. Although it was found to be slower in forming hard tissues and less successful in capping than MTA in this study, it led to formation of a dentin bridge similar to actual physiologic dentin in the quality of a calcific bridge. Actually, a dentin bridge, which contained dentinal tubules, differed from osteo-dentin when MTA was used.

DDP was less likely to close the pulp exposure area and formed hard tissues more slowly in pulp capping than MTA probably because of its imperfect properties. Bacteria-tight sealing makes MTA better than other materials and leads to more effective pulp capping. Further research should be conducted to improve sealing and operability of a DDP-using material for pulp capping and regenerative endodontic treatment in a true sense can be performed using DDP, which is more biocompatible than any other material.

In conclusion, using MTA for pulp capping can be more successful in forming a calcified bridge. However, DDP, a biocompatible material, can form a physiologic dentin bridge and is expected to become more useful as a pulp capping material if its fabrication is improved.

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**Conflict of Interest**

The authors have declared that no COI exists.

**References**
