Original

Effect of Direct Pulp Capping with a Novel Chemically Curable Mineral Trioxide Aggregate Material using Tri-Butylborane as a Polymerization Initiator

Chidzuru Inami1,2, Chihiro Endoh1, Hitoshi Ichinohe3 and Shinichi Itsuno3

1) Department of Applied Chemistry and Life Science, Toyohashi University of Technology, Toyohashi, Japan
2) Sun Medical Co., Ltd. Moriyama, Japan
3) Safety Research Institute for Chemical Compounds Co., Ltd. Sapporo, Japan

(Received for publication, October 16, 2019)

Abstract: Although mineral trioxide aggregate (MTA) has excellent sealing properties and biocompatibility and is currently widely used in various applications including direct pulp capping, several problems associated with its use, such as variable curing time and inconsistent physical properties, persist. We have developed a chemically curable, resin-modified type of MTA material (PCX-TBB) using tri-n-butylborane (TBB) as a polymerization initiator to improve the aforementioned problematic characteristics. We previously reported that PCX-TBB exhibited good physicochemical properties, excellent calcium releasing ability, and enhanced hard tissue induction ability compared with those of TheraCal LC® (Bisco, Schaumburg, IL, USA), which is already used clinically. In the present study, we further clarified the biocompatibility and healing ability of PCX-TBB as a direct pulp capping material. The histopathological changes when PCX-TBB and TheraCal LC® were applied to the wound-exposed pulps of dogs were investigated. We evaluated the grade of inflammatory cell infiltration and state of dentin bridge formation 7 days and 69 days after the application of PCX-TBB and TheraCal LC®. It was found that the inflammatory changes in the repair of cavities via pulp capping using PCX-TBB were the same as those observed with the use of TheraCal LC®. The dentin bridges formed 69 days after the application of both the materials, and the use of PCX-TBB resulted in a greater extent of dentin bridge formation in each cavity than that achieved by TheraCal LC®. The dentin bridges formed with the use of PCX-TBB were greater in area than that of the largest dentin bridge formed using TheraCal LC® at least 70% of the specimens. These findings suggest that PCX-TBB is more biocompatible and effective as a direct pulp capping material than TheraCal LC®.

Key words: Hard tissue-induction ability, Mineral trioxide aggregate, Tri-butylborane, Regeneration, Resin-modified

Introduction

The direct pulp capping material induces the formation of a dentin bridge by covering the exposed pulp. Mineral trioxide aggregate (MTA) was launched in 1998 as ProRoot® MTA (Dentsply Tulsa Dental, Tulsa, OK, USA) and has been used as a material with excellent sealing properties and biocompatibility in various applications, including direct pulp capping1–3. To date, various MTA-based products have been developed. The curing mechanisms of these products are based on the hydration reaction of Portland cement, which is the major component of MTA. These products suffer from several problems, such as variable curing time and inconsistent physical properties, owing to differences in the mixing ratio of powder and water4–9. Recently, a resin-modified type of pulp capping material composed of Portland cement and resin monomer, which addresses the abovementioned problems, has been made commercially available (TheraCal LC®, Bisco, Schaumburg, IL, USA). TheraCal LC® is a light-curable paste with improved operability compared with that of conventional MTA, and its calcium releasing ability, which is an important performance parameter for hard tissue induction, is superior to that of ProRoot® MTA7. Furthermore, it is reported that the hard tissue induction ability of TheraCal LC® is the same as that of Portland cement9.

We have developed a chemically curable, resin-modified type of MTA material (PCX-TBB) using tri-n-butylborane (TBB) as a polymerization initiator to improve the operability of the MTA base material. We evaluated the physicochemical properties of PCX-TBB in comparison with those of TheraCal LC®, which is already used clinically, and confirmed that PCX-TBB exhibited low solubility, high calcium releasing ability, and stable compressive strength9. Furthermore, it was revealed that PCX-TBB had improved calcium releasing and hard tissue induction abilities compared with those of TheraCal LC®.

Tri-n-butylborane, which is a polymerization initiator for PCX-TBB, has been used as a polymerization initiator for another dental adhesive material, Super-Bond (Sun Medical Co., Ltd., Moriyama, Japan). Although Super-Bond is a resin material, it exhibits high biocompatibility and good potential as a direct pulp capping material10,11. The excellent biocompatibility of Super-Bond likely originates from the unique polymerization mechanism initiated by TBB12,13. Since PCX-TBB too is polymerized by TBB, it can be expected to have high biocompatibility, equivalent to that of Super-Bond.

Therefore, this study was performed to examine the biocompatibility and healing ability of PCX-TBB, a novel, chemically curable MTA material, in comparison with those of TheraCal LC®, which is already used clinically, by investigating the histopathological changes in the
Materials and Methods

Materials
The materials used in this study as well as their manufacturer details and compositions are listed in Table 1.

Methods
This study was conducted according to the guidelines of the International Organization for Standardization (ISO7405:2008; Evaluation of biocompatibility of medical devices used in dentistry).

Criteria related to animal welfare
This study was conducted according to the following criteria: ISO 10993-2:2006: Biological evaluation of medical devices – Part 2: Animal welfare requirements, Act on Welfare and Management of Animals, Act No. 105, October 1, 1973; latest amendment, Act No. 51, June 2, 2014, and Standards Relating to the Care, Management and Refinement of Laboratory Animals, Notification No. 88 of the Ministry of the Environment (MOE), April 28, 2006; latest amendment, Notification No. 84 of the MOE, August 30, 2013.

Ethics of animal experimentation
This study was reviewed by the Animal Experimentation Ethics Committee of the Safety Research Institute for Chemical Compounds to verify the need for animal experimentation and mitigate suffering. This study was approved by the facility managers and was conducted in accordance with the testing facility’s code of ethics for animal experimentation (Authorization number: AN201710914-01).

Laboratory animals
The animals used were five 13–14-month-old female Nalc beagles (Kitayama Labes). The given material was applied to eight teeth, which were randomly selected from 10 teeth in total: the maxillary third premolar and first molar (on both sides), and the mandibular third premolar and first and second molars (on both sides). Each animal was fed 300 g of feed per day, and water was supplied via a water bottle ad libitum.

Cavity preparation and pulp capping
The analgesic meloxicam (0.04 ml/kg, Boehringer Ingelheim Japan Co., Ltd., Tokyo, Japan) was administered subcutaneously to the dogs, and then anesthesia was induced via intravenous administration of propofol. The dose of anesthetic was calculated for each animal based on its weight on the day of application of pulp capping material. The animals were intubated with an endotracheal tube, and anesthesia was maintained via inhalation of isoflurane (inhalation concentration 3.0–5.0%, Mylan Inc., Canonsburg, PA, USA). During surgery, normal saline (isotonic sodium chloride solution) was intravenously infused via the cephalic vein. A volume of 0.3–1.8 ml of lidocaine hydrochloride 2% (Xylocaine for dental, Dentsply Sirona Inc., Tokyo, Japan) with epinephrine was administered around the tooth to which a pulp capping material was applied, and infiltration anesthesia was performed. In addition, the surface of the tooth was disinfected with hydrogen peroxide and chlorhexidine (Ugaigusuri Kororo SP, Saraya Co., Ltd., Tokyo, Japan). After disinfection, a class V cavity was prepared in the buccal or lingual aspect of the center of the crown of the tooth using a diamond point (FG Reg 440SS, Shofu Inc., Kyoto, Japan). The pulp at the center of the cavity was exposed (0.6 mm in diameter) with a steel bur (Emil Lange, CA No.1/4, Shofu Inc., Kyoto, Japan) while the cavity was flushed with normal saline. The cavity was irrigated using normal saline, and bleeding was stopped. Next, the cavity was dried using sterile swabs. The PCX-TBB was prepared by mixing 0.1 g of the paste with a drop of Catalyst V, and was then applied to the exposed pulp. In accordance with the manufacturer’s instructions, TheraCal LC® was applied in a thickness of 1 mm or less to cover at least 1 mm of the dentin around the cavity, and it was light-cured for 20 s or longer using a dental light curing unit (D-Lux 10, Dentrade Ltd., Osaka, Japan) with a light intensity of 500 mW/cm². As a pulp capping material, PCX-TBB was applied to five cavities and TheraCal LC® was applied to three cavities per dog. The teeth to which PCX-TBB and TheraCal LC® were applied were randomly selected from the 10 teeth mentioned earlier, and the materials were applied in a random order. Once the pulp capping material was applied, the cavity was sealed with Bondfill SB Plus (Sun Medical Co., Ltd., Moriyama, Japan).

Specimen preparation
Seven days and 69 days after the pulp capping materials were applied, the analgesic meloxicam was administered subcutaneously to the dogs, who were then euthanized via exsanguination by severing the axillary artery under anesthesia with sodium pentobarbital (0.6 ml/kg, Kyoritsu Seiyaku Corp., Tokyo, Japan). Next, the fillings, teeth, and supporting tissues were observed macroscopically, and the maxilla and mandible were then collected. The maxilla and mandible were fixed in 10% neutral buffered formalin and stored. The teeth to which a test substance had been applied were decalcified, a paraffin block was prepared, and serial sections, 5–10 µm thick, spanning the total length of the cavity in the direction of the long axis of each tooth were prepared. Next, a hematoxylin-eosin-stained (HE-stained) specimen was prepared from each section.

Assessment of pulp
The pulp exposure owing to cavity preparation and changes in the pulp were observed during the day of application of each pulp capping material.

Grading of inflammatory cell infiltration
For each tooth to which a pulp capping material was applied, five specimens at even intervals along the total length of the cavity were selected and examined microscopically. The inflammatory cell infiltration of the pulp tissue adjacent to the exposed pulp was graded according to ISO7405:2008 (Table 2). The points were tallied and divided by the number of graded specimens to serve as the average grade for each tooth. An inflammatory response index was determined based on the average grades of all teeth to which a material was applied.

Extent of dentin bridge formation
For each tooth to which a pulp capping material was applied, five specimens at even intervals along the total length of the cavity were identified. The extent of bridge formation was assessed in the specimens with the greatest extent of dentin bridge formation as determined visually. The extent of bridge formation was graded three grades according to ISO 7405: 2008: Grade 0 indicating no formation of dentin bridge, Grade 1 indicating partial formation, and Grade 2 indicating complete sealing of pulp tissue.

Area of dentin bridge
Among the specimens with dentin bridge formation, those with the
One specimen with the greatest extent and one with the least extent of dentin bridge formation were selected, and the areas of the regions of bridge formation were calculated using the following method.

1) A photograph was taken at a magnification at which the entire region of dentin bridge formation was within the field of view. A stage micrometer was imaged at the same magnification used during specimen imaging.

2) A 5 mm grid was applied to each photograph obtained in 1).

3) Squares, at least three-quarters occupied by dentin bridge, were visually identified in the photograph with a 5 mm square grid, and the number of those squares (\(x\)) was tallied.

4) The length (mm: \(y\)) of the stage micrometer scale used to view a developed negative film was measured.

5) The long axis of the photograph in 1) and the long axis of its negative film were measured, and the magnification factor of the photograph was calculated \([\text{long axis of photograph in mm}/\text{long axis of negative film in mm}: z]\).

6) The values of \(x\), \(y\), and \(z\) obtained above were substituted into Equation 1, and the area of the region of dentin bridge formation was calculated.

\[
\text{Area of the region of dentin bridge formation (μm}^2\) = \(x \times [5/(y \times z)]^2 \times 10^6
\]

**Distribution of dentin**

The inner edge of the dentin before pulp exposure was judged from the intensity of HE-stained. The pulp cavity was defined as the area between the inner edge of the dentin and the closest root canal dentin, and the distribution of dentine bridge occupying the entire pulp cavity was determined by the following grading. The extent to which dentin filled the pulp cavity was graded from 0 to 4, with Grade 0 indicating that dentin has not entered the pulp cavity, Grades 1, 2, and 3 indicating that dentin occupies up to one-quarter, one-half, and three-quarters, respectively, of the pulp cavity, and Grade 4 indicating that dentin occupies almost the entire pulp cavity or has spread.

**Results**

The grade of inflammatory cell infiltration in the pulp tissue adjacent to the pulp expose and the state of dentin bridge formation 7 days and 69 days after the application of PCX-TBB and TheraCal LC® are shown in Tables 3 and 4, respectively. A representative histology is shown in Figs. 1 to 3.

**Assessment of pulp exposure**

(7 days after application)

Pulp exposure owing to cavity preparation was noted in nine of the 10 cavities to which PCX-TBB was applied and five of the six cavities to which TheraCal LC® was applied. Necrosis of the pulp was not noted with either material. Of the 10 cavities to which PCX-TBB was applied, slight or mild inflammatory cell infiltration was noted in one (10.0%) and a foreign body reaction by macrophages was noted in six (60.0%). Of the six cavities to which TheraCal LC® was applied, slight or mild inflammatory cell infiltration of the pulp was noted in two (33.3%) and a slight foreign body reaction by macrophages was noted in one (16.7%). A representative histology of inflammatory cell and the foreign body reaction by macrophages is shown in Fig. 1.
Table 3. Histopathological evaluation of PCX-TBB and TheraCal LC® (7 days after application)

| Material     | Animal No | Implanted part (Molar) | Grades of inflammatory cells in the pulp tissue adjacent to the pulp exposure | Forming of dentin bridge |
|--------------|-----------|-------------------------|-----------------------------------------------------------------------------|---------------------------|
|              |           |                         | Grades of inflammation (Average value of five specimens)                    | Index of inflammatory reaction | Grade | Distribution | Area (µm²) |
|              |           |                         |                                                                             |                           |        |             | (Animal’s No.-Part) |
| PCX-TBB      | 1         | UL No.4 0                | 0                                                                            | 0 (–)                     | 0 (-)  | 0.2          | (-)         |
|              |           | LR No.4 0                | 0                                                                            | 0 (–)                     | 0 (-)  |             |             |
|              |           | LR No.5 *                | 0                                                                            | 0 (–)                     | 0 (-)  |             |             |
|              |           | LL No.4 0                | 2                                                                            | 0 (–)                     | 0 (-)  |             |             |
|              | 2         | UL No.5 0                | 0                                                                            | 0 (–)                     | 0 (-)  |             |             |
|              |           | LR No.4 0                | 0                                                                            | 0 (–)                     | 0 (-)  |             |             |
|              |           | LR No.6 0                | 0                                                                            | 0 (–)                     | 0 (-)  |             |             |
|              |           | LL No.4 0                | 0                                                                            | 0 (–)                     | 0 (-)  |             |             |
| TheraCal LC® | 1         | UL No.4 0                | 0                                                                            | 0 (–)                     | 0 (-)  | 0.0          | (-)         |
|              |           | LR No.6 0                | 0                                                                            | 0 (–)                     | 0 (-)  |             |             |
|              |           | LR No.6 *                | 0                                                                            | 0 (–)                     | 0 (-)  |             |             |
|              | 2         | UL No.4 0                | 0                                                                            | 0 (–)                     | 0 (-)  |             |             |
|              |           | LR No.6 0                | 0                                                                            | 0 (–)                     | 0 (-)  |             |             |
|              |           | LL No.4 0                | 0                                                                            | 0 (–)                     | 0 (-)  |             |             |

* The number shows the position from a front molar. UR: Upper right, LR: Lower right, UL: Upper left, LL: Lower left
b 0: No inflammation, 1: Mild inflammation, 2: Moderate inflammation, 3: Severe inflammation, 4: Abscess formation or extended inflammatory cell infiltration not limited only to the pulp tissue adjacent to the pulpal exposure
c A total number of the averages for inflammation grades /The number of graded teeth (PCX-TBB: 9 teeth, TheraCal LC®: 5 teeth)
d 0: No formation of dentin bridge, 1: Partial formation, 2: Complete sealing of pulp
e 0: Dentin bridge is formed except the exposed pulp area, **: Dentin bridge is formed except the exposed pulp area, (-): Blank

Table 4. Histopathological evaluation of PCX-TBB and TheraCal LC® (69 days after application)

| Material     | Animal No | Implanted part (Molar) | Grades of inflammatory cells in the pulp tissue adjacent to the pulp exposure | Forming of dentin bridge |
|--------------|-----------|-------------------------|-----------------------------------------------------------------------------|---------------------------|
|              |           |                         | Grades of inflammation (Average value of five specimens)                    | Index of inflammatory reaction | Grade | Distribution | Area (µm²) |
|              |           |                         |                                                                             |                           |        |             | (Animal’s No.-Part) |
| PCX-TBB      | 3         | UR No.5 0                | 0                                                                            | 0 (–)                     | 0 (-)  | 0.6          | Minimum: 20,000 (No.5-UR No.4) Maximum: 379,000 (No.5-LL No.4) |
|              |           | UL No.4 0                | 2                                                                            | 1                         | 1      |             |             |
|              |           | LR No.4 3                | 1                                                                            | 1                         | 2      |             |             |
|              |           | LR No.6 0                | 2                                                                            | 1                         | 2      |             |             |
|              | 4         | UL No.5 2                | 1                                                                            | 1                         | 2      |             |             |
|              |           | UR No.4 0                | 1                                                                            | 1                         | 2      |             |             |
|              |           | LR No.6 0                | 1                                                                            | 1                         | 3      |             |             |
|              |           | LL No.4 0                | 2                                                                            | 1                         | 3      |             |             |
|              | 5         | LR No.5 *                | 2                                                                            | 1                         | 4      |             |             |
|              |           | LL No.5 **               | **                                                                          |                           |        |             |             |
| TheraCal LC® | 3         | UL No.5 0                | 0                                                                            | 0 (–)                     | 0 (-)  | 1.0          | Minimum: 21,000 (No.5-LR No.5) Maximum: 54,000 (No.3-LR No.5) |
|              |           | LR No.5 0                | 1                                                                            | 1                         | 1      |             |             |
|              | 4         | LR No.6 0                | 1                                                                            | 1                         | 1      |             |             |
|              |           | LL No.4 1                | 1                                                                            | 1                         | 1      |             |             |
|              |           | LR No.5 0                | 0                                                                            | 0 (–)                     | 0 (-)  |             |             |

* The number shows the position from a front molar. UR: Upper right, LR: Lower right, UL: Upper left, LL: Lower left
b 0: No inflammation, 1: Mild inflammation, 2: Moderate inflammation, 3: Severe inflammation, 4: Abscess formation or extended inflammatory cell infiltration not limited only to the pulp tissue adjacent to the pulpal exposure
c A total number of the averages for inflammation grades /The number of graded teeth (PCX-TBB: 13 teeth, TheraCal LC®: 7 teeth)
d 0: No formation of dentin bridge, 1: Partial formation, 2: Complete sealing of pulp
e 0: Dentin bridge is formed except the exposed pulp area, **: Dentin bridge is formed except the exposed pulp area, (-): Blank
Chidzuru Inami et al.: Effect of Direct Pulp Capping with a Novel Chemically Curable MTA

Pulp exposure owing to cavity preparation was observed in 13 of the 14 cavities to which PCX-TBB was applied and seven of the eight cavities to which TheraCal LC® was applied. Necrosis of the pulp was not noted with either material. Of the 14 cavities to which PCX-TBB was applied, slight or moderate inflammatory cell infiltration was noted in three (21.4%) and dentin bridges had formed in all 14 cavities (100.0%). Of the eight cavities to which TheraCal LC® was applied, slight or mild inflammatory cell infiltration of the pulp was noted in four (50.0%) and dentin bridges had formed in seven (87.5%). A foreign body reaction by macrophages was not noted with either material.

(69 days after application)

Pulp exposure owing to cavity preparation was observed in 13 of the 14 cavities to which PCX-TBB was applied and seven of the eight cavities to which TheraCal LC® was applied. Necrosis of the pulp was not noted with either material. Of the 14 cavities to which PCX-TBB was applied, slight or moderate inflammatory cell infiltration was noted in three (21.4%) and dentin bridges had formed in all 14 cavities (100.0%). Of the eight cavities to which TheraCal LC® was applied, slight or mild inflammatory cell infiltration of the pulp was noted in four (50.0%) and dentin bridges had formed in seven (87.5%). A foreign body reaction by macrophages was not noted with either material.

**Grading of inflammatory cell infiltration**
(7 days after application)
The inflammatory cell infiltration of the pulp tissue adjacent to the exposed pulp was graded in nine of the 10 cavities with exposed pulp to which PCX-TBB was applied and five of the six cavities to which TheraCal LC® was applied. The inflammatory response index was 0.2 for PCX-TBB and 0.0 for TheraCal LC®.

(69 days after application)
The inflammatory cell infiltration of the pulp tissue adjacent to the exposed pulp was graded in 13 of the 14 cavities with exposed pulp to which PCX-TBB was applied and seven of the eight cavities to which TheraCal LC® was applied. The inflammatory response index was 0.6 for PCX-TBB and 1.0 for TheraCal LC®.

**Extent of dentin bridge formation**
(7 days after application)
Dentin bridges had not formed with the application of either PCX-TBB or TheraCal LC®.
(69 days after application)
With PCX-TBB, dentin bridges had formed on the exposed pulp in 13 of the 14 cavities; in the remaining cavity, the dentin bridge had formed beyond the exposed pulp. The dentin bridge on the exposed pulp was of Grade 1 (the dentin bridge was partially present on the exposed pulp) in eight cavities and of Grade 2 (the exposed pulp had been completely sealed with the dentin bridge) in five cavities. With TheraCal LC®, the dentin bridges had formed on the exposed pulp in seven of the eight cavities, and all were of Grade 1 (the dentin bridge was partially present on the exposed pulp).

**Area of dentin bridge**
(7 days after application)
Dentin bridges on exposed pulps were not noted with either PCX-TBB or TheraCal LC®.
(69 days after application)
A comparative analysis of the amount of dentin bridge formation indicated that in 10 of the 14 cavities to which PCX-TBB was applied, the amount of dentin formed was greater than the maximum amount of dentin formed with the application of TheraCal LC®. The area of dentin was 20,000–379,000 µm² when PCX-TBB was applied and 21,000–54,000 µm² when TheraCal LC® was used.

**Distribution of dentin bridge**
(7 days after application)
Dentin bridges on the exposed pulps were not noted with either PCX-TBB or TheraCal LC®.
(69 days after application)
The distribution of dentin in the 14 cavities to which PCX-TBB was applied was as follows: Grade 1 (dentin occupied up to one-quarter of the pulp cavity) in six cavities, Grade 2 (dentin occupied about one-half of the pulp cavity) in five cavities, Grade 3 (dentin occupied up to three-quarters of the pulp cavity) in two cavities, and Grade 4 (dentin occupied almost the entire pulp cavity or had spread beyond the flat por-
Figure 3. Histopathological images of PCX-TBB and TheraCal LC® (69 days after implanted, Black arrows indicates the dentin bridge). A: PCX-TBB (Animal No.3, UR No.5, Grades of inflammation 0: No inflammation, Forming of dentin bridge 1: Partial formation, Distribution of dentin bridge 1: Dentin bridge occupies up to 1/4 of the pulp cavity), B: PCX-TBB (Animal No.3, LR No.6, Grades of inflammation 0: No inflammation, Forming of dentin bridge 2: Complete sealing of pulp, Distribution of dentin bridge 2: Dentin bridge occupies about 1/2 of the pulp cavity), C: PCX-TBB (Animal No.3, LL No.5, Grades of inflammation 3: Severe inflammation, Forming of dentin bridge 1: Partial formation, Distribution of dentin bridge 1: Dentin bridge occupies up to 1/4 of the pulp cavity), D: TheraCal LC® (Animal No.5, UR No.5, Grades of inflammation 0: No inflammation, Forming of dentin bridge 1: Partial formation, Distribution of dentin bridge 1: Dentin bridge occupies up to 1/4 of the pulp cavity), E: TheraCal LC® (Animal No.3, LR No.5, Grades of inflammation 0: No inflammation, Forming of dentin bridge 1: Partial formation, Distribution of dentin bridge 2: Dentin bridge occupies about 1/2 of the pulp cavity), F: TheraCal LC® (Animal No.5, LR No.5, Grades of inflammation 2: Moderate inflammation, Forming of dentin bridge 1: Partial formation, Distribution of dentin bridge 1: Dentin bridge occupies up to 1/4 of the pulp cavity).
tion of the cavity) in one cavity. The distribution of dentin in seven of the eight cavities to which TheraCal LC® was applied was as follows: Grade 1 (dentin occupied up to one-quarter of the pulp cavity) in six cavities, and Grade 2 (dentin occupied about one-half of the pulp cavity) in one cavity.

**Discussion**

Direct pulp capping material is used at the site of the exposed pulp during the removal of carious tooth substance or cavity preparation, and the emphasis is on the biological healing of the pulp. Calcium hydroxide, which has often been used as a direct pulp capping material, promotes dentin bridge formation. However, it has drawbacks; for instance, a necrotic layer forms directly below the exposed pulp, and dead space is created between the pulp capping material and new dentin bridge. In contrast, MTA seldom triggers an inflammatory response even though it is highly alkaline; furthermore, it is highly biocompatible and provides exceptional sealing. Hence, it has garnered attention as a direct pulp capping material to replace calcium hydroxide. The reparative and healing effects of MTA presumably originate from calcium hydroxide, which is produced when calcium silicate (a key ingredient of Portland cement) reacts with water. In other words, MTA must be in contact with water to be therapeutically effective. Many of the MTA materials in current clinical use involve the mixing of a powder containing MTA with water. The MTA comes into contact with water during the preparation of the material. In contrast, TheraCal LC®, the light-curable pulp capping material used in this study, is a paste containing MTA and is directly applied to the exposed pulp, and hence, MTA does not come into contact with water during the preparation of the material. Polyethylene glycol dimethacrylate (Table 1), a monomer contained in TheraCal LC®, is highly hydrophilic. Once the material is applied to the exposed pulp, the tissue fluid in the exposed pulp readily permeates the material. The tissue fluid reacts with MTA, which presumably accounts for the therapeutic effectiveness of TheraCal LC®. The new, chemically cured, pulp capping material, i.e., PCX-TBB, contains hydroxypropyl metacrylate, which is a hydrophilic monomer (Table 1). It reacts with the tissue fluid in the exposed pulp, resulting in a therapeutic effectiveness similar to that of TheraCal LC®.

In the current study, cavities with exposed pulp were prepared in the teeth of dogs and were directly capped with PCX-TBB or TheraCal LC®. Their effectiveness as direct pulp capping materials was assessed 7 days and 69 days after application. During the test, there were no changes observed in the general condition of the dogs, their weights, and macroscopic findings in the mouths related to the application of either material.

The index for an inflammatory response in the pulp tissue adjacent to the exposed pulp 7 days after application was 0.2 for PCX-TBB and 0.0 for TheraCal LC®, indicating that the use of PCX-TBB resulted in a higher inflammatory response index. However, the inflammatory response occurred only in one of the nine cavities to which PCX-TBB was applied, and changes indicative of serious inflammation were not noted (Table 3, Fig. 2).

The index for an inflammatory response in the pulp 69 days after application was 0.6 for PCX-TBB versus 1.0 for TheraCal LC®. There were no changes indicative of serious inflammation (Table 4, Fig. 3). When observed 69 days after application, the pulp was found completely sealed with PCX-TBB in about 36% of the overall teeth tested; no teeth lacked dentin bridge. When observed 69 days after the application of TheraCal LC®, the dentin bridges had formed but the exposed pulps were not completely sealed. When a dentin bridge forms, the presence of tunnel defects reportedly hampers its effectiveness as a barrier. In the present study, tunnel defects were not noted with either material.

The dentin bridges formed with the application of both materials were observed; those formed using PCX-TBB appeared thicker than those formed using TheraCal LC®, and hence, the areas of the dentin bridges were assessed and compared. The results indicated that the dentin bridges formed using PCX-TBB were bigger than the largest dentin bridge formed using TheraCal LC® (54,000 µm²) in at least 70% of the specimens. A comparison of the dentin bridge distribution in the cavities revealed that 57% of the cavities occupied one-half to all of the pulp cavity when PCX-TBB was used. The use of PCX-TBB yielded significantly improved results than those obtained with the use of TheraCal LC® (14%).

In ISO7405: 2008, it is recommended that the thickness and range of dentin bridge formation should be examined. If dentin bridge formation is incomplete, the exposed pulp cannot be protected effectively. On the contrary, if a large amount of dentin bridge is formed randomly during healing, the pulp cavity would occlude, which will cause a risk that the activity of the pulp decreases. In this study, the state of dentin bridge formed by PCX-TBB was superior to that of TheraCal LC®. With regard to the distribution of dentin bridge, the use of PCX-TBB resulted in dentin bridge formed inside the pulp cavity without occluding the pulp cavity. These findings suggest that PCX-TBB is more biocompatible and effective as a direct pulp capping material than TheraCal LC®.

A crucial aspect of the biocompatibility of MTA is the sustained release of calcium ions and hydroxide ions produced by calcium hydroxide as a result of hydration. As mentioned above, TheraCal LC® and PCX-TBB contain a hydrophilic monomer, and hence, the tissue fluid in the exposed pulp readily penetrates the pulp capping material, where it reacts with the MTA in the material. This explains why TheraCal LC® releases more calcium over time than that released by ProRoot MTA®. The calcium ions released by MTA over time react with the phosphate ions in the tissue fluid, forming apatite-like crystals of calcium phosphate on the surface of the MTA material. Kokubo et al. reported that the examination of the apatite formed on the surface of a material immersed in a simulated bodily fluid (SBF) could effectively predict bone regeneration in vivo. We found that PCX-TBB had a greater ability to form apatite in SBF than that of TheraCal LC®, suggesting that PCX-TBB may display an exceptional ability to induce dentin formation in vivo. The current findings agree with the results of the previous study.

According to the manufacturer’s instructions, TheraCal LC® should be applied in a thickness of 1 mm or less and should be light-cured. However, when the materials are prepared for experiments, the presence of an unpolymerized layer after light curing has been noted. The pulp capping treatment takes place in an environment with a large amount of moisture and oxygen and where it is harder to deliver light, and hence, it is more difficult to ensure that the material is polymerized during treatment than during material preparation for an experiment. Nevertheless, TBB activates polymerization more than that activated by the typical polymerization initiators used in dentistry, and it is additionally reported to initiate greater polymerization activity in the presence of a certain amount of oxygen and water. The PCX-TBB was more biocompatible than TheraCal LC® in the current study; this may be owing to the unique properties of TBB as a polymerization initiator.

In conclusion, the inflammatory changes in the repair of cavities via pulp capping using PCX-TBB, a novel, chemically curable, resin-modified type of MTA material, were the same as those observed with the use of TheraCal LC®, a light-curable MTA material, which is already used...
clinically. It is suggested that PCX-TBB can promote the formation of dentin bridge more successfully than that achieved by TheraCal LC®. The present study demonstrates that PCX-TBB has high biocompatibility and clinical utility in comparison with those of a pulp capping material already in clinical use.

Conflicts of Interest
The materials costs relevant to this study were borne by Sun Medical Co., Ltd.

Author Chidzuru Inami is an employee of Sun Medical Co., Ltd. and applied for a patent related to this research, but has not received any patent royalty.

There are no other conflicts of interest to disclose.

References
1.  Okiji T and Yoshida K. Reparative Dentinogenesis Induced by Mineral Trioxide Aggregate: A Review from the Biological and Physicochemical Points of View. Int J Dent 2009: doi: 10.1155/2009/464280
2.  Parirlokh M and Torabinejad M. Mineral trioxide aggregate: A comprehensive literature review - Part III: Clinical applications, drawbacks, and mechanism of action. J Endod 36: 400-413, 2010
3.  Torabinejad M and Chivian N. Clinical applications of mineral trioxide aggregate. J Endod 25: 197-205, 1999
4.  Torabinejad M, Hong CU, McDonald F and Pitt Ford TR. Physical and chemical properties of a new root-end filling material. J Endod 21: 349-353, 1995
5.  Chiang TY and Ding SJ. Comparative physicochemical and bio-compatible properties of radiopaque dicalcium silicate cement and mineral trioxide aggregate. J Endod 36: 1683–1687, 2010
6.  Basturk FB, Nekoofar MH, Gunday M and Dummer PM. Effect of varying water-to-powder ratios and ultrasonic placement on the compressive strength of mineral trioxide aggregate. J Endod 41: 531-534, 2015
7.  Gandolfi MG, Siboni F and Prati C. Chemical-physical properties of TheraCal, a novel light-curable MTA-like material for pulp capping. Int Endod J 45: 571-579, 2012
8.  Cannon M, Gerodias N, Viera A, Percinoto C and Jurado R. Primate pulpal healing after exposure and TheraCal application. J Clin Pediatr Dent 38: 333-337, 2014
9.  Inami C, Nishitani Y, Haraguchi N and Itsuno S. Evaluation of the solubility, calcium-release ability, and apatite-forming ability of a novel chemically curable mineral trioxide aggregate material. J Hard Tissue Biol 28: 273-279, 2019
10. Imaizumi N, Kondo H, Ohya K, Kasugai S, Araki K and Kurosaki N. Effects of exposure to 4-META/MMA-TBB resin on pulp cell viability. J Med Dent Sci 53: 127-133, 2006
11. Nakamura M, Inoue T and Shimono M. Immunohistochemical study of dental pulp applied with 4-META/MMA-TBB adhesive resin after pulpotomy. J Biomed Mater Res 51: 241-248, 2000
12. Inoue T and Shimono M. Repair dentinogenesis following transplantation into normal and germ-free animals. Proc Finn Dent Soc 88: 183-194, 1992
13. Nakagawa K, Saita M, Ikeda T, Hirota M, Park W, Lee MC and Ogawa T. Biocompatibility of 4-META/MMA-TBB resin used as a dental luting agent. J Prostheth Dent 144: 114-121, 2015
14. Hirabayashi C and Imai Y. Studies on MMA-TBB resin. I. Comparison of ThB and other initiators in the polymerization of PMMA/MMA resin. Dent Mater J 21: 314-321, 2002
15. Kwon TY and Imai Y. Polymerization characteristics of ethyl methacrylate-based resin initiated by TBB. Dent Mater J 23: 161-165, 2004
16. Taira Y and Imai Y. Review of methyl methacrylate (MMA)/tributylborane (TBB)-initiated resin adhesive to dentin. Dent Mater J 33: 291-304, 2014
17. Inami C, Shimizu H, Suzuki S, Haraguchi N and Itsuno S. Study on the performance of methyl methacrylate polymerization: Comparison of partially oxidized tri-n-butylborane and benzoyl peroxide with aromatic tertiary amines. Dent Mater J 38: 430-436, 2019
18. Cox CF, Subay RK, Ostro E, Suzuki S and Suzuki SH. Tunnel defects in dentin bridges: Their formation following direct pulp capping. Oper Dent 21: 4-11, 1996
19. Fujitani M, Shibata S, Van Meerbeek B, Yoshida Y and Shintani H. Direct adhesive pulp capping: Pulpal healing and ultra-morphology of the resin-pulp interface. Am J Dent 15: 395-402, 2002
20. Koike T, Polan MA, Izumikawa M and Saito T. Induction of reparative dentin formation on exposed dental pulp by dentin phosphophoryn/collagen composite. Biomed Res Int 2014: doi: 10.1155/2014/745139
21. Mohammadi Z and Dummer PM. Properties and applications of calcium hydroxide in endodontics and dental traumatology. Int Endod J 44: 697-730, 2011
22. Torabinejad M, Hong CU, Pitt Ford TR and Kaiyawasam SP. Tissue reaction to implanted super-EBA and mineral trioxide aggregate in the mandible of guinea pigs: a preliminary report. J Endod 21: 569-571, 1995
23. Ozdemir HO, Ozcilik B, Karabucak B and Cehreli ZC. Calcium ion diffusion from mineral trioxide aggregate through simulated root resorption defects. Dent Traumatol 24: 70-73, 2008
24. Koh ET, McDonald F, Pitt Ford TR and Torabinejad M. Cellular response to Mineral Trioxide Aggregate. J Endod 24: 543-547, 1998
25. Kokubo T and Takada H. How useful in SBF in predicting in vivo bone bioactivity? Biomater 27: 2907-2915, 2006
26. Chen L and Suh BI. Cytotoxicity and biocompatibility of resin-free adhesive based on partially oxidized tributylborane (TBB). Dent Mater J 23: 161-165, 2004
27. Okamoto Y, Takahata K and Saeki K. Studies on the behavior of partially oxidized tributylborane as a radical initiator for methyl methacrylate (MMA) polymerization. Chem Lett 27: 1247-1248, 1998.