# Evaluation of a Bioceramic as a Pulp Capping Agent *In Vitro* and *In Vivo*

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## Abstract

**Introduction:** This study aims to investigate the effects of the bioceramic iRoot BP Plus (Innovative Bioceramix Inc, Vancouver, Canada) as a pulp capping agent *in vitro* and *in vivo*. **Methods:** *In vitro*, human dental pulp cells (hDPCs) were seeded into plates with the prepared iRoot BP Plus or mineral trioxide aggregate (MTA) packed in the bottom of different wells. The proliferation of hDPCs was determined using the 3,(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide assay. Meanwhile, 2 animal models of direct pulp capping and pulpotomy were applied in Wistar rats *in vivo*. The exposed pulps were capped with iRoot BP Plus or MTA. After 1 and 4 weeks, maxillary segments were obtained and prepared for histologic analysis. **Results:** hDPCs grew very well even in the place contacted with MTA or iRoot BP plus *in vitro*. MTA and iRoot BP Plus both enhanced the proliferation of hDPCs ($P < .05$). *In vivo*, results revealed that few inflammatory cells were present in the pulpal area corresponding to the pulp exposure. A slight layer of newly generated matrix was also observed next to MTA and iRoot BP Plus after 1 week. A complete reparative dentin bridge with polarizing odontoblastlike cells was detected in all specimens in the iRoot BP Plus group after 4 weeks. **Conclusions:** iRoot BP Plus exhibited good biocompatibility to pulp tissue and induced the proliferation of dental pulp cells and the formation of reparative dentin bridge. iRoot BP plus may be used as a pulp capping material for vital pulp therapy *(J Endod 2015;41:652–657)*



## Key Words

Bioceramic, biocompatibility, cell proliferation, dental pulp capping, pulpotomy, reparative dentin


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The selection of a pulp capping material is an important factor that influences the success of vital pulp therapy. An ideal pulp capping material must possess good biocompatibility and strong antibacterial activity. It should also be capable of inducing the differentiation of dental pulp cells and the formation of reparative dentin.

The widely used conventional pulp capping agent is calcium hydroxide. It was considered the gold standard pulp capping agent (1). However, calcium hydroxide exhibits poor physical properties and incomplete dentin bridge formation, with tunnel defects that may lead to the failure of pulp capping (2–4). Mineral trioxide aggregate (MTA) has been recently recommended as a potential pulp capping material. MTA presents higher biocompatibility and sealing ability than calcium hydroxide (5). MTA can also induce the differentiation of dental pulp cells to odontoblastlike cells and form thicker dentin bridges (6–8). Aguilar and Linsuwanont (9) clinically compared the weighted pooled success rate of direct pulp capping using $Ca(OH)_2$ or MTA as the pulp capping material; they showed that MTA demonstrated a more successful outcome than calcium hydroxide. However, MTA still has some limitations, including difficult handling characteristics and long setting time.

iRoot BP Plus (Innovative Bioceramix Inc, Vancouver, Canada) is a newly developed calcium silicate–based bioactive ceramic. This material is a convenient and ready-to-use white premix in putty form. iRoot BP Plus not only exhibits satisfactory biocompatibility (10–12), sealing ability (13, 14), and antibacterial activity (15, 16) but also up-regulates the expression of mineralization-related genes (17, 18). Hence, iRoot BP Plus is a potential pulp capping material. However, information about this material remains lacking. This study aims to assess the effects of this bioceramic iRoot BP Plus as a pulp capping agent *in vitro* and *in vivo*.

## Materials and Methods

### *In Vitro* Assessment

**Cells and Cell Culture Conditions.** Human dental pulp cells (hDPCs) were originally obtained from impacted third molars or premolars that were extracted for orthodontic purposes from patients aged 18–26 years old at the Peking University School and Hospital of Stomatology. Primary pulp cells were harvested through enzyme digestion with 3 mg/mL collagenase type I (Sigma-Aldrich, St Louis, MO). The cells were expanded and cultured in Dulbecco modified Eagle medium (DMEM; Gibco, Gaithersburg, MD) supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin. All cells were cultured at 37°C in 5% $CO_2$ and 95% humidity. The cells used in this study were obtained from the fourth to sixth passages.

**Material Preparation and Cell Seeding.** Samples (2 mm in diameter and 1 mm in height) of iRoot BP Plus and MTA (Dentsply Tulsa Dental, Tulsa, OK) were prepared in sterile plastic molds according to the manufacturer's instructions. The materials were immediately packed in the bottom of different wells of 12-well plates under sterile conditions and allowed to solidify at 37°C in 5% $CO_2$ and 95% humidity for 24 hours before exposure to hDPCs. Then, hDPCs were seeded on 12-well plates that contain the as-prepared materials at a density of $5 \times 10^4$ cells per well in 2 mL DMEM. The control group was cultured with DMEM without any material. At 1, 3, 5, and 7 days postseeding, the cells were collected for proliferation assay.

**MTT Assay.** The proliferation of hDPCs was determined using the 3,(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT)-based colorimetric assay. As described previously, cells were seeded into 12-well plates without any

material, with iRoot BP Plus, or with MTA. After 1, 3, 5, and 7 days, 100 $\mu$L MTT solution (Amresco, Solon, OH) was added to each well, and the cells were incubated for an additional 3 hours. The resulting MTT formazan crystals were dissolved by removing the culture medium and adding 500 $\mu$L dimethyl sulfoxide (Amresco) to each well. The plate was shaken at room temperature for 10 minutes to dissolve the crystals. Then, 100 $\mu$L of the solution in each well was transferred to a 96-well plate for absorbance determination at 570 nm with a microplate reader (ELX808; BioTek, Winooski, VT).

**Statistical Analysis.** Experiments were performed in triplicates. Data were expressed as the mean ± standard error of the number of observations. SPSS13.0 (SPSS Inc, Chicago, IL) was used for statistical testing. Results were analyzed using 1-way analysis of variance, and $P < .05$ was considered significant.

### *In Vivo* Assessment

Twenty male Wistar rats weighing about 180–200 g were used for *in vivo* study. Twelve rats were used for direct pulp capping and the remaining 8 rats for pulpotomy. All experimental procedures were performed in accordance with the animal experimental guidelines of Peking University Health Science Center.

**Direct Pulp Capping Assay.** Twenty-four maxillary first molars were obtained from the 12 rats and randomly divided into 3 groups: negative control, iRoot BP Plus, and MTA. The rats were anesthetized with an intraperitoneal injection of 2% pentobarbital. After cleaning and disinfecting the teeth with cotton soaked in 75% ethanol, class V cavities were prepared on the mesial surfaces of the maxillary first molar with 0.6-mm-diameter round burs. To avoid pulp impairment from heat during cavity preparation, the teeth and cutting instruments were irrigated with sterile distilled water. Pulps were exposed with the tip of a #15 sterile stainless steel file through the remaining thin dentin of each cavity. Bleeding was weak, and hemostasis was performed by pressing a sterile saline cotton pellet for a few seconds. The pulp perforation sites were directly capped with iRoot BP Plus or MTA in the 2 experimental groups, whereas the pulp was not capped with any pulp capping agent in the control group. 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 broken to minimize occlusal forces.

**Pulpotomy Assay.** Sixteen maxillary first molars were obtained from the 8 rats and randomly divided into 2 groups (ie, negative control and iRoot BP Plus groups). After anesthetization, the maxillary first molars of the rats were excavated from the occlusal surface to the pulp chamber by using high-speed sterile 0.6-mm-diameter round burs with water cooling. Coronal pulp was removed, and hemorrhage was controlled by pressing a sterile saline cotton pellet. In the experimental group, iRoot BP Plus was placed on the exposed pulp, and the cavity was sealed with the glass ionomer cement Fuji IX. In the negative control group, the cavity was directly filled with glass ionomer without using any pulp capping agent. The cusp tips of the opposing teeth were worn down.

**Sample Preparation and Histologic Analysis.** The animals were anesthetized and sacrificed at 1 and 4 weeks after direct pulp capping and pulpotomy. The maxillary sections, including 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 then embedded in paraffin. The sections with a thickness of 5 $\mu$m were cut in a mesiodistal direction for hematoxylin-eosin staining and Masson trichrome (Baso Diagnostic Inc, Zhuhai, Guangdong, China) staining according to the manufacturer's protocol.

Histologic features were evaluated according to the criteria presented in Table 1 (19–21) by 2 observers who were not informed of the true nature and purpose of the study. Inflammatory cell response and hard tissue formation were evaluated using scores of 1 to 4.

## Results

### Effect of iRoot BP Plus on the Proliferation of hDPCs

In general, hDPCs grew very well even in the place contacted with MTA or iRoot BP Plus. The MTT assay was used to investigate the proliferation of hDPCs (Fig. 1). The proliferation of hDPCs in the MTA group was enhanced on days 5 and 7, whereas iRoot BP Plus increased cell numbers on days 1 to 7. iRoot BP Plus significantly enhanced the proliferation of hDPCs on days 1 and 3 compared with MTA ($P < .05$).

### Effect of iRoot BP Plus on the Formation of Dentin Bridge *In Vivo*

A satisfactory interobserver agreement was obtained in the histologic evaluation. The evaluation scores of each group after direct pulp capping and pulpotomy are presented in Table 2 and summarized as follows.

#### Direct Pulp Capping Results.

***1-Week Observation Period.*** In the control group, one quarter of the specimens exhibited mild inflammatory response, and the remaining three quarters showed moderate inflammatory response. No hard tissues were observed at the site of pulp exposure in any of the specimens (Fig. 2*A-1*, *A-2*, and *A-3*).

In the MTA and iRoot groups, the inflammatory cell response scores were similar after 1 week. About three quarters of the specimens exhibited no or very few inflammatory cells, and one quarter showed a mild inflammatory response in the pulpal area that corresponded to pulp exposure. Three quarters of the specimens in the MTA group presented a slight layer of newly generated matrix next to the material (Fig. 2*B-1*, *B-2*, and *B-3*), whereas all specimens in the iRoot group exhibited mild hard tissue deposition (Fig. 2*C-1*, *C-2*, and *C-3*).

**TABLE 1.** Criteria Used for the Histologic Analysis of the Pulps Treated with Direct Pulp Capping and Pulpotomy: Inflammatory Cell Response and Hard Tissue Formation

| Grade | Inflammatory cell response (direct pulp capping/pulpotomy) | Hard tissue formation |
|---|---|---|
| 1 | Absent or very few inflammatory cells | Heavy hard tissue deposition as a complete dentin bridge |
| 2 | Mild: inflammatory cells only next to dentin bridge or area of pulp exposition/reaching up to one third of the root canal pulp tissue | Moderate hard tissue deposition |
| 3 | Moderate: inflammatory cells are observed in the part of coronal pulp/up to two thirds of the root canal pulp tissue | Only a slight layer of hard tissue deposition |
| 4 | Severe: all coronal pulp/more than two thirds of the root canal pulp tissue is infiltrated or necrotic | No hard tissue deposition |

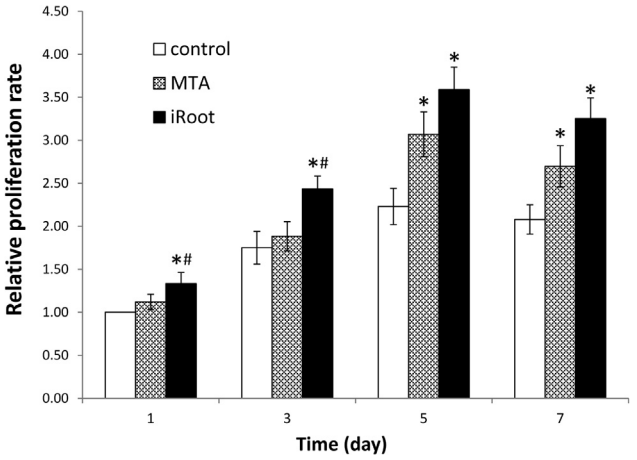


**Figure 1.** The effects of iRoot BP Plus and MTA on the proliferation of hDPCs as obtained using the MTT assay. *Significant differences from the control values with $P < .05$. #Significant differences from the MTA values with $P < .05$.

***4-Week Observation Period.*** After 4 weeks, necrosis occurred from the site of pulp exposure to almost whole pulp tissue in all specimens of the control group (Fig. 2*D-1*, *D-2*, and *D-3*). One section of the control group showed incomplete hard tissue deposition below the exposed area (Fig. 2*D-3*). One half of the specimens in the control group did not exhibit hard tissue deposition.

Reparative dentin bridges were directly observed at the injury site in three quarters of the specimens in the MTA group (Fig. 2*E-1*, *E-2*, and *E-3*) and in all specimens in the iRoot BP Plus group (Fig. 2*F-1*, *F-2*, and *F-3*). The newly formed reparative dentin was connected to the primary dentin and contained homogenous dentinal tubulelike structures. Polarizing odontoblastlike cells aligned along this layer, and the adjacent pulp tissue appeared normal without inflammatory cells. In 1 specimen in the MTA group, the dentin bridge was incomplete, and inflammatory cells appeared next to the reparative dentin.

### Pulpotomy Results

***1-Week Observation Period.*** About three quarters of the specimens in the control group exhibited a moderate inflammatory infiltration of the radicular pulp, and the remaining one quarter showed a mild inflammatory response. No hard tissues were observed in all specimens in the control group. All specimens in the iRoot BP Plus group presented a layer of hard tissues formed at the canal orifice, and the pulp tissue under the material in three quarters of the specimens exhibited no inflammatory cells, which is characteristic of normal tissue (Fig. 3*A-1* and *A-2*).

***4-Week Observation Period.*** After 4 weeks, the pulp tissue in the root canal was completely infiltrated or necrotic in all specimens in the control group, and almost no hard tissue was observed. Three quarters of the specimens in the iRoot BP Plus group presented a thick layer of dentin bridge, which completely sealed the canal orifice. Polarizing odontoblastlike cells were observed below the dentin bridge, and the radicular pulp lacked inflammatory cells (Fig. 3*B-1* and *B-2*). Only 1 specimen in the iRoot group exhibited a moderate inflammatory infiltration and incomplete reparative dentin bridge.

## Discussion

This study confirmed that the newly developed calcium silicate–based bioactive ceramic known as iRoot BP Plus can induce the formation of reparative dentin bridge at the site of mechanically exposed rat pulps subjected to direct pulp capping and pulpotomy. Considering that direct pulp capping and pulpotomy are main methods in vital pulp therapy, we used them in this study to evaluate iRoot BP Plus as a pulp capping material. Besides *in vivo* assessment, *in vitro* study was also performed to evaluate the biocompatibility of iRoot BP Plus on the pulp tissue. Thus far, most studies on iRoot BP Plus focused on its characteristics as a root canal repair material or root canal sealer by using cell models of fibroblast cultures (10, 11, 22), osteoblast cultures (17, 23), and periodontal ligament cells (24). In the present *in vitro* study, we selected hDPCs as the cell model and placed the materials in the bottom of cell-culture wells to ensure direct contact with the cells and thus more accurately simulate the clinical condition.

The results revealed that iRoot BP Plus exhibited good biocompatibility to the pulp tissue. After capping the exposed pulp tissue with iRoot BP Plus, few inflammatory cells were detected. Furthermore, the *in vitro* MTT assay showed that MTA and iRoot BP Plus promoted the proliferation of hDPCs. iRoot BP Plus also enhanced the proliferation higher than the MTA. This finding may be caused by the fine hydrophilic calcium silicate content of iRoot BP Plus. iRoot BP Plus is a ready-to-use putty substance that does not require mixing; this material primarily consists of calcium silicates, calcium phosphate, zirconium

**TABLE 2.** Grading of the Histologic Sections of the Pulps Subjected to Direct Pulp Capping and Pulpotomy

| | | Inflammatory cell response score | | | | | Hard tissue formation score | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Observation period | No. of specimens | 1 | 2 | 3 | 4 | * | 1 | 2 | 3 | 4 | * |
| Direct pulp capping | | | | | | | | | | | |
| 1 week | | | | | | | | | | | |
| Control | 4 | 0 | 1 | 3 | 0 | 2.75 | 0 | 0 | 0 | 4 | 4.00 |
| MTA | 4 | 3 | 1 | 0 | 0 | 1.25 | 0 | 0 | 3 | 1 | 3.25 |
| iRoot BP Plus | 4 | 3 | 1 | 0 | 0 | 1.25 | 0 | 0 | 4 | 0 | 3.00 |
| 4 weeks | | | | | | | | | | | |
| Control | 4 | 0 | 0 | 0 | 4 | 4.00 | 0 | 1 | 1 | 2 | 3.25 |
| MTA | 4 | 3 | 1 | 0 | 0 | 1.25 | 3 | 1 | 0 | 0 | 1.25 |
| iRoot BP Plus | 4 | 4 | 0 | 0 | 0 | 1.00 | 4 | 0 | 0 | 0 | 1.00 |
| Pulpotomy | | | | | | | | | | | |
| 1 week | | | | | | | | | | | |
| Control | 4 | 0 | 1 | 3 | 0 | 2.75 | 0 | 0 | 0 | 4 | 4.00 |
| iRoot BP Plus | 4 | 3 | 1 | 0 | 0 | 1.25 | 0 | 0 | 4 | 0 | 3.00 |
| 4 weeks | | | | | | | | | | | |
| Control | 4 | 0 | 0 | 0 | 4 | 4.00 | 0 | 0 | 1 | 3 | 3.75 |
| iRoot BP Plus | 4 | 3 | 0 | 1 | 0 | 1.50 | 3 | 1 | 0 | 0 | 1.25 |

*Means for each group in each sub item of the criteria.

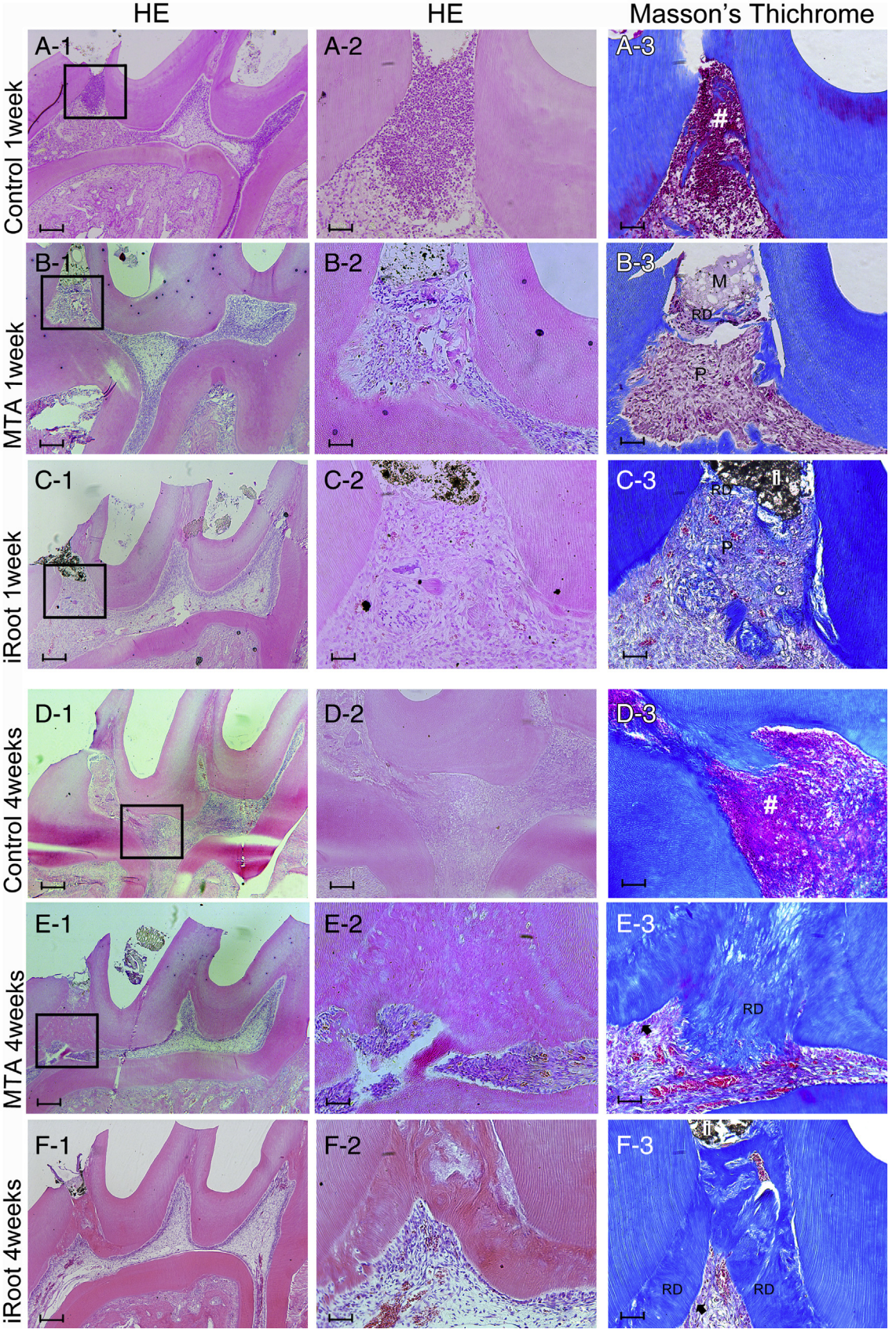


**Figure 2.** Hematoxylin-eosin and Masson trichrome staining evaluation of the effects of MTA and iRoot BP Plus on direct pulp capping assay. *A-2* and *A-3* represent the high magnification of *A-1*, *B-2* and *B-3* represent the high magnification of *B-1*, and so on. M, MTA; i, iRoot BP Plus; P, pulp; #, inflammatory or necrosis area; RD, reparative dentin; *arrow*, odontoblastlike cells. Scale bar of *A-1*, *B-1*, *C-1*, *D-1*, *E-1*, and *F-1* = 200 $\mu$m. Scale bar of *A-2*, *B-2*, *C-2*, *D-2*, *E-2*, *F-2*, *A-3*, *B-3*, *C-3*, *D-3*, *E-3*, and *F-3* = 50 $\mu$m.

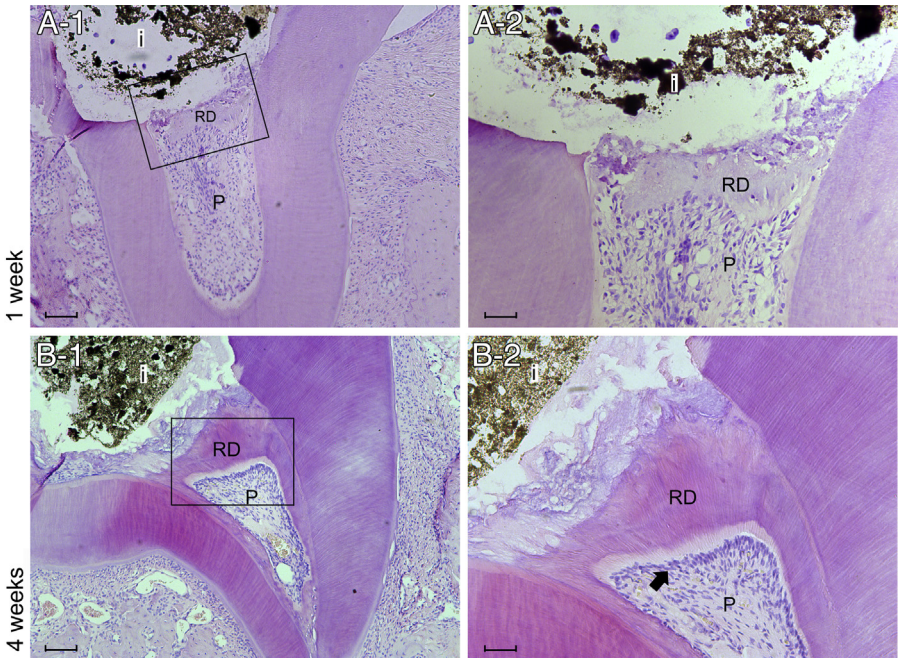


**Figure 3.** Histologic features of pulpotomy with iRoot BP Plus as the pulp capping material (*A-1* and *A-2*) after 1 week and (*B-1* and *B-2*) 4 weeks. *A-2* and *B-2* represent the high magnification of *A-1* and *B-1*, respectively. i, iRoot BP Plus; P, pulp; RD, reparative dentin; arrow, odontoblastlike cells. Scale bar of *A-1* and *B-1* = 100 $\mu$m. Scale bar of *A-2* and *B-2* = 50 $\mu$m.

oxide, and tantalum oxide. When the material comes in contact with the naturally present moisture in the dentinal tubules, calcium silicates reacts with $H_2O$ to produce calcium silicate hydrogel and calcium hydroxide; calcium hydroxide reacts with calcium phosphate to form hydroxyapatite and $H_2O$. $H_2O$ is supposed to start the reaction cycle again (25, 26). The high levels of calcium and silicon might explain the good biocompatibility of iRoot BP Plus (17). MTA and iRoot BP Plus have a similar composition; however, MTA contains bismuth oxide as the radiopacifier, whereas iRoot BP Plus comprises tantalum oxide (27). Bismuth oxide may reduce the biocompatibility of MTA compared with iRoot BP Plus (28).

This study showed that iRoot BP Plus can induce the formation of reparative dentin bridge. This induction capability of iRoot BP Plus was observed earlier and was stronger than that of MTA. The exposed pulp sites with iRoot BP Plus and MTA exhibited similar inflammatory cell response scores at 1 week after direct pulp capping. A layer of newly generated matrix was observed next to the material in three quarters of the specimens in the MTA group and in all specimens in the iRoot BP Plus group. After 4 weeks, a thick layer of dentin bridge formed at the exposed site, and odontoblastlike cells aligned along this layer in all specimens in the iRoot group. The dentin bridge induced by iRoot BP Plus was continuous and contained no pores, thus effectively sealing the exposed site. The dentin bridge in one quarter of the specimens in the MTA group was incomplete. However, the effects of these 2 materials on inducing the reparative dentin bridge formation should be investigated using more large sample studies.

Some studies also investigated and compared the effect of iRoot BP Plus with that of MTA on dental pulp cells. Zhang et al (18) found that iRoot BP Plus significantly up-regulated the activity of alkaline phosphatase (ALP) and the expression of dentin sialophosphoprotein (DSPP), dentin matrix protein-1 (DMP-1), and osteocalcin (OCN) of hDPCs; these genes are associated with mineralization and odontoblastic differentiation (29, 30). The enhancement of iRoot BP Plus was also significantly higher than that of MTA (18). Another study indicated that iRoot BP Plus exhibited excellent apatite-forming capability *in vitro*, and the extracts of iRoot BP Plus promoted the adhesion, migration, and attachment of DPSCs; these processes are the key phenomena during the initial stage of dental pulp repair (31). The stronger apatite-forming capability of iRoot BP Plus than MTA may be attributed to the differences in their components, particle sizes, and bioactivity (31). Our results were consistent with these 2 reports. However, these studies used the extracts of iRoot BP Plus instead of directly applying the material. More studies are needed to elucidate the mechanism of action of iRoot BP Plus on pulp tissue.

In summary, iRoot BP Plus promoted the proliferation of hDPCs and induced the formation of reparative dentin bridge at the exposed pulp site *in vivo*. Given its good bioactivities and optimal handling characteristics, iRoot BP Plus can be used as a pulp capping material for vital pulp therapy.

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*The authors deny any conflicts of interest related to this study.*

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