ORIGINAL ARTICLE

Dentin Regeneration by Direct Pulp Capping Using a Bioabsorbable Material

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SYNOPSIS
The aim of this study was to investigate the effect of bioabsorbable material on the direct pulp capping to know whether this material has a function in dentin regeneration by bioabsorption.

Class V cavities with the exposed pulp area were prepared on the buccal surface of first molars in young beagle dogs. All cavities were conditioned with a self-etching primer (Clearfil SE Primer). In the cavities of right side (experimental group), the exposed pulp was covered with a bioabsorbable sheet (Kurabio AG), and then the cavities were coated with an adhesive agent (Clearfil SE Bond) and resorted with a resin composite. The left side cavities (control group) were applied the adhesive agent without the bioabsorbable sheet and restored with a resin composite. On three and six months after surgery, the teeth were extracted, immersion-fixed and decalcified. Afterward, the teeth were frozen-sectioned, stained with hematoxylin and eosin and observed using a light microscopy.

In the experimental group, a large amount of the tertiary dentin was formed into the prepared cavity. On the other hand, the tertiary dentin was almost not formed into the cavity in the control group.

The significantly tertiary dentin formation was recognized into the prepared cavity when the exposed pulp area had been covered by a bioabsorbable material. This result suggested that the space making by a bioabsorbable material could produce the derivation of pulp tissue and then dentin regeneration into the prepared cavity.

Key words: bioabsorbable material, tertiary dentin, regeneration, direct pulp capping

INTRODUCTION
Deep caries treatment occasionally causes a pulp exposure and therefore drives to do the direct pulp capping to the exposed pulp. Recently, the adhesive resin systems are used as a direct pulp capping material. It is reported that the adhesive resin system provided an acceptable pulpal inflammatory and dentinal bridge repair responses, comparable with those of calcium hydroxide. On the other hand, numerous researchers reported that the adhesives did not allow the complete connective tissue repair adjacent to the pulp exposure site. Unpolymerized resin monomer has the cytotoxicity and inhibits the mineral nodule formation.
Therefore, it is better that a mediator which has a high biocompatibility has been placed on the exposed pulp prior to the application of the adhesive resin.

Calcium hydroxide is commonly used for the direct pulp capping and promptly induces the tertiary dentin which is classified as a reparative dentin formed by a new generation of odontoblast-like cells. However, this tertiary dentin which is called the dentinal bridge arises toward the pulp chamber from the exposed pulp area and is not formed from the exposed pulp area to the prepared cavity. Briefly, the lost dentin is never reconstructed by the natural dentin and thus replaced by dental materials as an artificial dentin. Whereas, Kitasako et al. and Arakawa et al. had reported that a protrusion of pulp tissue into the prepared cavity was observed at the periphery of the exposed area and then an irregular dentin bridge was formed when the adhesive resin systems was used for the direct pulp capping. These findings have suggested that the derivation of pulp tissue into the prepared cavity may achieve a natural dentinal reconstruction at the space of the lost dentin. However, it is difficult that nonabsorbable material like an adhesive resin can make an adequate space for derivation of pulp tissue into the cavity.

Alginate wound dressings can promote the tissue regeneration by maintaining a moist condition and bioabsorption in wounds. Moreover, these alginate gels are unsuitable for bacterial culture medium and can relatively resist the bacterial infection. It has been reported that numerous alginate wound dressings release large amount of calcium ions and be cytotoxic to the cells. On the other hand, newly developed freeze-dried alginate gel dressing contains a low calcium concentration and shows no cytotoxic effect on fibroblasts. This freeze-dried alginate gel dressing may work as a bioabsorbable material and make space for derivation of pulp tissue into the cavity when the exposed pulp area was covered by this wound dressing.

In this study, we investigated the effect of bioabsorbable material on the direct pulp capping to know whether this material has a function in dentin regeneration by bioabsorption.

**MATERIALS AND METHODS**

Four young female beagle dogs (11-12 months) were used in this study. Dogs were housed in facilities approved by the Okayama University and kept according to the ethical guidelines for animal care in Okayama University (OKU-2005125). The dogs were placed under general anesthesia by intramuscular injection of 20 mg/kg Ketamine (Ketalar 50, Sankyo Yell Yakuhin Co., Ltd, Tokyo, Japan) and intravenous injection of 20 mg/kg pentobarbital sodium bodyweight (Nembutal, Dainippon Pharmaceutical Co., Ltd, Osaka, Japan). Local infiltration of 1.8 ml of 2% Xylocaine with 1:80,000 epinephrine in the buccal vestibules was delivered to supplement the anesthesia.

Class V cavities, standardized at 5 mm in diameter and 5 mm above the gingival margin, were prepared on the buccal surface of the first molars using a high speed tapered diamond bur (ISO#170/016) under water spray coolant. The pulps were exposed with a #1 carbide bur (0.8 mm in diameter) on the cavity floor. The cavities were chemically irrigated with 3% hydrogen peroxide and 6% sodium hypochlorite, and then rinsed with normal saline.

After hemostasis, all of the cavity walls including the exposed pulp were conditioned with a self-etching primer (Clearfil SE Primer, Kuraray Medical Co., Ltd, Tokyo, Japan) for 20 s and then gently air-dried. In the cavities of right side, the exposed pulp was covered with
a thinned bioabsorbable sheet (Kurabio AG, Kuraray Medical), 1mm × 1mm in width, and then the cavities were coated with an adhesive agent (Clearfil SE Bond, Kuraray Medical), which was light-cured for 10 s (Kurabio AG group) (Fig. 1). Resin composite (Clearfil AP-X, Kuraray Medical) was applied to the cavity and light-cured for 30 s. The cavities of left side were directly applied the adhesive agent and resin composite to the exposed pulp area without the bioabsorbable sheet (Control group).

On three and six months after surgery, the dogs were sacrificed with an overdosage of Nembutal. The teeth were immediately extracted and immersion-fixed with Zamboni’s fixative (2% paraformaldehyde and 0.2% picric acid in 0.1 mol/L phosphate buffer, pH 7.4) and decalcified in 4.17% buffered disodium dihydrogen ethylenediamine tetra-acetate dihydrate (pH 7.4) for 4 weeks at room temperature. The teeth were then immersed in 20% sucrose in phosphate buffered saline (PBS, pH 7.4) for 24 h, frozen-sectioned at 20 μm thickness and mounted on silane-coated slides (Dako, Kyoto, Japan).

Sections of the teeth were stained with hematoxylin and eosin (HE) and dehydrated using a graded series of alcohols, cleared in xylene and coverslipped with Entellan (Merck, Darmstadt, Germany). For pathological observation, a light microscopy (BX40, Olympus Co., Ltd., Tokyo, Japan) was used.

The area of the tertiary dentin formed into the prepared cavity was also measured. Five serial sections with the exposed pulp per tooth were selected and these images were captured with a digital microscopic system (DP-12 and BX40, Olympus Co., Ltd.). These images were analyzed with Scion image software (http://www.scioncorp.com/) and the area of the tertiary dentin formed into the cavity was measured. The data per tooth was averaged and the mean value was used as the area of the tertiary dentin of each tooth. The data was statistically analyzed using Tukey-Kramer test at a significance level of 0.05.

RESULTS
For the Kurabio AG group a large amount of the tertiary dentin was formed.
This tertiary dentin was classified into the external tertiary dentin, which was formed from the exposed pulp area to the prepared cavity, and the internal tertiary dentin formed from the exposed pulp area to the pulp cavity (Fig. 2 and Table 1). Fig. 3 shows the typical microphotographs for the Kurabio AG material.

Fig. 2 Classification of the formed tertiary dentin

Fig. 3 Microphotographs for experimental group with Kurabio AG

Fig. 4 Microphotographs for control group
a: the section with an exposed pulp area, b: the section without an exposed pulp area, ITD: Internal tertiary dentin, EP: Exposed pulp area, CA: Prepared cavity
Table 1  Number of tooth with the external/external tertiary dentin formation

<table>
<thead>
<tr>
<th></th>
<th>Kurabio AG group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 months</td>
<td>2/2 (2)</td>
<td>2/2 (2)</td>
</tr>
<tr>
<td>6 months</td>
<td>3/3 (4)</td>
<td>2/4 (4)</td>
</tr>
</tbody>
</table>

Parenthetic number shows the number of specimen in each group. A specimen in the experimental group at 6-month was excluded for reticular atrophy.

Table 2  Area of the external tertiary dentin formed into the prepared cavity ($\times 10^3 \mu m^2$)

<table>
<thead>
<tr>
<th></th>
<th>Kurabio AG group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 months</td>
<td>56.4±7.1</td>
<td>1.2±0.9*</td>
</tr>
<tr>
<td>6 months</td>
<td>223.5±114.8</td>
<td>24.0±40.0*</td>
</tr>
</tbody>
</table>

The asterisks within each group indicate no statistically significant difference (Tukey-Kramer test, p=0.05).

Fig. 5  The area of the external tertiary dentin in each group

The external tertiary dentin made relatively extensive contact with the prepared cavity wall (Fig. 3a). In addition, this dentin was obviously observed in a neighboring section without an exposed pulp area (Fig. 3b).

On the other hand, external tertiary dentin was almost not formed in the prepared cavity for the control group even after 6 months, although the internal tertiary dentin was formed for all teeth (Table 1 and Fig. 4a). In a neighboring section without an exposed pulp area, external tertiary dentin was never observed in the prepared cavity (Fig. 4b).

Fig. 5 and Table 2 show the area of the external tertiary dentin in each group. Kurabio AG group was significantly larger area of the tertiary dentin than the control group at 3 and 6 months (p<0.05). For Kurabio AG group the area of the dentin after 6 months was significantly larger than that after 3 months (p<0.05). On the other hand, there was no significant difference in the area of the external tertiary dentin for
the control group between 3 and 6 months, though the mean area of the dentin at 6 month was larger than that at 3 months.

DISCUSSION
In the present study, we investigated that the formation of the tertiary dentin at a nearby exposed pulp area covered with or without a bioabsorbable material. For the Kurabio AG group with a bioabsorbable material the significant formation of the tertiary dentin, which was classified into the internal and external tertiary dentin, was observed. On a direct pulp capping, the exudates from the exposed pulp into the cavity along the wall prevent the sealing between the restorative material and the tooth substrate. This poor sealing makes a gap and then causes the protrusion of the pulp tissue by the pulpal internal pressure. Thereafter, the extruded pulp tissue produces an irregular tertiary dentin formation at the periphery of the cavity. On the other hand, when the alginate wound dressing was used for the cover to the exposed pulp, the sealing between the adhesive and cavity wall was excellent because this dressing can absorb the exudates. In addition, this material which absorbed the tissue fluid can keep the wetting environment which enhances the healing. Since this tissue fluid contains much kind of growth factors for wound healing, the healing of the exposed pulp tissue has to be a speedy recovery. After that, it is likely that the space as a result of the bioabsorption was filled with a connective pulp tissue migrated from the pulp chamber. As this migrated pulp cells are including the undifferentiated mesenchymal stem cells, these cells can differentiate into the odontoblast-like cells. A marked external tertiary dentin observed in this study suggests to be formed by these differentiated odontoblast-like cells. It is undoubtedly that the odontoblast-like cells migrate toward the pulp chamber with the formation of the external tertiary dentin and then form the internal tertiary dentin in the pulp chamber. Thus, the internal tertiary dentin recognized in the Kurabio AG group is bound to be formed by not only these odontoblast-like cells but also surviving post-mitotic odontoblast cells stimulated by the cavity preparation.

On the other hand, in the control group which was directly covered by an adhesive resin, the obvious external tertiary dentin was almost devoid except for an irregular tertiary dentin at the periphery of the cavity. The polymerized adhesive resin can not be absorbed biologically and not make a sufficient space for migration of pulp tissue. As a result of unabsorbable adhesive layer, it is likely that the external tertiary dentin was almost not formed in the cavity. Although the external tertiary dentin formation was inhibited in the control group, the internal tertiary dentin was formed. It is considered that this dentin was produced by the newly differentiated odontoblast-like cells and surviving post-mitotic odontoblast cells as well as the Kurabio AG group. However, the pulp directly contacts to the unpolymerized resin monomers when the adhesive resin system is applied to the exposed pulp. It is reported that the unpolymerized resin monomer had the cytotoxicity and inhibited the mineral nodule formation. Moreover, adhesive resin induces the apoptosis and cell-cycle arrest of pulp cells. These findings suggest that the direct pulp capping agent using an adhesive resin delays the wound healing and prevent the tertiary dentin formation at the exposed pulp tissue. From the point of view the employment of the adhesive system may be unfavorable for the direct pulp capping agent.

In conclusion, the marked tertiary dentin formation was recognized into
the prepared cavity when the exposed pulp area had been covered by a bioabsorbable material, Kurabio AG. However, the tertiary dentin was almost not observed into the cavity which was directly applied the adhesive system to the exposed pulp area. These results suggested that the space making by a bioabsorbable material could produce the derivation of pulp tissue and then dentin regeneration into the prepared cavity.

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