The Usefulness of New Hydroxyapatite as a Pulp Capping Agent in Rat Molars

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Abstract
To evaluate the usefulness of new hydroxyapatite (HAP) as a pulp capping agent, comparisons with a calcium hydroxide agent (calcital®) and formocresol, which are generally used for pulpotomy, were performed. Serial changes in pH in these materials were investigated. Furthermore, after pulpotomy in rat molars using each material, the degree of the formation of dentine-like hard tissue and inflammatory changes in the pulp tissue were micro CT radiographically and pathologically examined. It has been reported that calcital® was a strong alkali (pH 12) causes a high level of pulp irritation, which were same as our result. The new sintered hydroxyapatite was almost neutral in pH, with a low level of pulp irritation. Furthermore, as a result of micro CT radiographical and histopathological evaluation, dentin-like hard tissue formation underneath the sectioned pulp surface was most marked in the calcital® group, and no dentin-like hard tissue formation underneath the sectioned pulp surface was noted in the HAP group. However, the HAP group developed no necrotic layers, and inflammation improved at an earlier stage. Our experiments suggested that HAP can be a good pulp capping agent, since pulp irritation was lower with HAP than with calcital® and formocresol.

Introduction
It is well known in clinical pediatric dentistry that pulpotomy is useful for preserving deciduous and juvenile permanent tooth pulp without inflammation or injury (1). Pulpotomy is indicated for deciduous teeth whose root resorption is less than 1/2 of the tooth root, and juvenile permanent teeth with immature roots, in which inflammation is localized in the coronal pulp (2).

Although both calcium hydroxide and formocresol are generally used for pulpotomy, calcium hydroxide is more frequently used as a pulp capping agent in clinical cases. Calcium hydroxide has an excellent hard tissue inductibility; however, it occasionally causes internal pulp resorption (3), and makes root canal treatment difficult due to ectopic calcified bodies (4). Various pulp capping agents have been developed to solve these problems, focusing on materials which are less harmful to the pulp tissue than materials evoking strong biological irritation. Furthermore, in addition to the development of new pulp capping agents, a new pulp capping method using a laser has been reported (5, 6). As a new pulp capping agent, calcium phosphate has been reported. Since calcium phosphate has been applied as a bone filling material and used in artificial tooth root cases, calcium phosphate materials are biologically excellent materials (7, 8, 9).

In this study, we evaluated the usefulness of hydroxyapatite, in which calcium phosphate with a
good bio-affinity is used as the base material, of a pulp capping agent to replace calcium hydroxide and formocresol.

**Materials and Methods**

*Materials used*

As the specimen material, hydroxyapatite 100% (HAP) was sintered by spark plasma sintering (SPS), using calcium phosphate (Sangi Co.) as the base material, and the specimen was ground into powder using a mortar. As the control materials, calvital (CV) (Neo Seiyaku Co.) as a calcium hydroxide agent, and pulpack V (Nippon Shika Yakuhin Co.) as a formocresol agent (FC) were selected, and each material was mixed at a powder-liquid ratio according to the manufacturer’s instructions. Furthermore, to evaluate changes in pH, non-sintered powder calcium phosphate was also used as a control.

*Changes in pH of surrounding pulp capping agents*

Powdered HAP (1 mg) and its base material, calcium phosphate (1 mg), were mixed with sterilized distilled water, respectively. CV was mixed at a powder-liquid ratio according to the manufacturer’s instructions. Each specimen was preserved in a polyethylene tube of 15 mm in internal diameter and 40 mm in depth at 25 °C. Changes in pH were serially measured after 0, 10, and 30 minutes, and 1, 3, 6, 24, and 48 hours, pouring distilled water into the polyethylene container. The mean value of 3 measurements was calculated in each specimen.

*Evaluation of bio-affinity*

1) Pulpotomy

Using 36 Wistar strain rats (3-week-old males, weighing 30–60 g), pulpotomy in the bilateral first molars was performed. The intraperitoneal administration of a mixture of ketamine hydrochloride (Ketalar, Sankyo Yell Yakuhin Co.) and xylazine hydrochloride (Celactal, Bayer Co.) (Ketalar : Celactal =18 : 5, 0.23 mg/100 g rat weight) was carried out. Pulpotomy was performed using No. 1/2 round burs, pouring sterilized physiological saline according to clinical procedures, under the simple exclusion of moisture.

After pulpotomy, irrigation was performed alternately using NaOCl (NeoCleaner, Neo Seiyaku Co.) and H$_2$O$_2$, and after confirming hemostasis by lightly applying a sterilized cotton ball, the wound surface of the sectioned pulp was capped with each specimen material. After pulp capping, cavities were prepared and filled with polycarboxylate cement (HY-BOND, Shofu Co.) and light-curing resin (Filtek Flow, 3M Co.).

2) Evaluation

Radiographical evaluation using a micro CT, and histopathology evaluation were performed. Micro CT (R-mCT, Rigaku Co.) was taken to evaluate serial changes 3 days, 1 and 2 weeks, and 1 month after pulpotomy under the conditions of tube voltage: 80 KV, tube current: 200 mA, image thickness: less than 0.125 mm, and Pixel size 20 μm. Regarding histopathology evaluation, rats were killed using CO$_2$ 3 days, 1 and 2 weeks, and 1 month after pulpotomy, and the maxillary bone with the bilateral first molars were immediately excised. After immersion and fixation in 10% neutral formalin, decalcification was performed using 10% EDTA solution for 14 days. Thereafter, paraffin embedding was performed according to conventional methods (10), and approximately 5 μm thick serial sections were cut. After hematoxylin–eosin staining, the presence of dentin-like hard tissue and inflammation was investigated using a light microscope.

**Results**

*Changes in pH of surrounding pulp capping agents*

Changes in pH in pulp capping agents are shown in Fig. 1. The mean pH in HAP was 6.87 (0 minutes), and 6.90 (10 minutes), showing a slight increase, and then 6.86 (30 minutes), 6.89 (1 hour), and around 6.8 (until 2 days), constantly showing values near neutrality. The mean pH in calcium phosphate was around 6.87 (0 minutes–4 hours), 6.80 (5 hours), showing a decrease, and around 6.88 (6 hours–2 days). The mean pH in calvital was 12.87 (0 minutes), 12.85 (30 minutes), and 12.89 (3 hours), showing an increase, and no changes were noted thereafter.
after surgery (Fig. 4), and formation of dentin-like hard tissue underneath the sectioned pulp surface was found 1 week after surgery (Fig. 5). Increases in the amount of dentin-like hard tissue formation were confirmed 2 weeks and 1 month after surgery (Figs. 6 and 7). The FC group showed no dentin-like hard tissue formation even 1 month after surgery (Fig. 8).

2) Histopathological findings

Table 1 shows dentin-like hard tissue formation...
and pulp inflammation underneath the pulp capping agents. At 3 days after surgery the HAP group showed inflammatory changes such as vasodilatation and leukocyte infiltration in the dental pulp. Although necrotic layers were confirmed, no formation of dentin-like hard tissue was detected (Fig. 9).

After 1 week after surgery, the HAP group indicated slightly inflammatory cells infiltration into the dental pulp underneath the pulp capping agent. However, inflammation decreased compared with 3 days after surgery, and the formation of dentin-like hard tissue was observed (Fig. 10). After 2 weeks, inflammation was resolved and the formation of dentin-like hard tissue on the pulpal wall increased compared with 1 week after surgery (Fig. 11). After 1 month, necrotic layers and inflammatory changes in the dental pulp underneath the sectioned surface

Table 1. Dentin-like hard tissue formation and pulp inflammation underneath the pulp capping agents

<table>
<thead>
<tr>
<th>Inflammation</th>
<th>Formation of dentin-like hard tissue (underneath pulp capping)</th>
<th>Formation of dentin-like hard tissue (the pulpal wall)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAP</td>
<td>3d</td>
<td>1w</td>
</tr>
<tr>
<td>Calvital</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Palpak</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

– ~ +: Not inflammation or Not dentin-like hard tissue ~ Much inflammation or Much dentin-like hard tissue
disappeared, and the formation of dentin-like hard tissue on the pulpal wall was noted (Fig. 12).

CV and FC groups showed similar findings from 3 days to week although in the both groups, the formation of necrotic layers underneath the sectioned pulp surface was confirmed, in the CV group, the formation of dentin-like hard tissue underneath the sectioned pulp surface was observed 2 weeks after surgery. The hard tissue formation was also simultaneously detected in the pulpal wall area in the CV group (Fig. 13).

In the FC group, the necrosis extended in the root apical direction, and no hard tissue formation was confirmed (Fig. 14).

**Discussion**

1. Evaluation of pulp irritation by pulp capping agents and changes in pH

Since deciduous teeth involve physiological tooth root resorption, it is desirable to avoid apical tooth treatment such as pulpectomy, and to maintain the tooth root pulp and periapical tissue under healthy conditions (11, 12). Pulpotomy is useful for this purpose, and the calcium hydroxide method and FC method are used. The purpose of the calcium hydroxide method is to leave a healthy root pulp by sectioning any infected coronal pulp, and to induce the cicatrical healing of the wound in the sectioned pulp (11), in which odontoblasts differentiate and proliferate from the dental pulp underneath the sectioned surface, and secondary dentin formation is facilitated to cover the sectioned surface with newly generated dentin (dentin bridge), maintaining a healthy root pulp (11). However, it was reported that calcium
hydroxide frequently caused abscess formation, and
the premature dislodgement of treated teeth due to
internal resorption (11). On the other hand, in the FC
method, no dentin–like hard tissue formation under-
neath the sectioned pulp surface is found, half the
root pulp on the root canal orifice side is fixed and
necrotized by formalin, and half the root pulp on the
root canal apical side is alive or replaced by granula-
tion tissue (11). There have been reports that the
success rate of the FC method was 70–80%, without
influences on the physiological resorption of the
deciduous tooth roots, although the tooth exchange
period was accelerated (11). The reason for the fre-
cquent use of calcium hydroxide for pulpotomy is that
a strong alkali causes coagulative necrosis in the
pulp surface layer, and this irritation induces dentin
formation and restoration in the pulp tissue (11). Our
results in this study showed that the pH in calvital
was constantly over 12, showing a high value and
high level of pulp irritation. On the other hand, the
pH in HAP was constantly near neutral, showing a
low level of pulp irritation in contrast to CV. As
described above, a strong alkali is necessary for
dentin–like hard tissue formation underneath the
sectioned pulp surface (3), and CV was the most
effective for dentin bridge formation underneath the
sectioned pulp surface in this study. However, our
results showed that the pH in HAP was constantly
around 6.8 in the neutral zone, suggesting that pulp
irritation is lower with HAP than with CV; there-
fore, HAP is less harmful to the body. Furthermore,
it was suggested that the pH in HAP was neutral in
accordance with that in calcium phosphate, which
was used as the base material of HAP, and the
elution of the pulp capping agent can be controlled
by changing the particle diameter without changing
the components using SPS; therefore, new pulp cap-
ping agents may be produced by mixing HAP with
other agents. Thus, sintered HAP can be a pulp
capping agent less harmful to the body than CV.

Evaluation of bio–affinity

Micro CT and histopathological findings showed
that inflammatory images appeared in every speci-
men 3 days after surgery. Furthermore, no specimen
showed dentin–like hard tissue on the third day.
Concerning calcium hydroxide and formocresol
agents, it has been reported that necrotic layers
containing blood clots and necrotic cells and tissue
were formed 2 days after pulpotomy (1), and this was
in accordance with our results. In our study, inflam-
matory images in HAP decreased 1 week after sur-
gery, and disappeared 2 weeks after surgery. These
results suggested that HAP causes less irritation to
the pulp, compared with CV and pulpack V®, which
have been conventionally used for pulpotomy.

As a result of the observation of serial changes in
dentin bridge formation for 1 month after surgery,
dentin–like hard tissue formation underneath the
sectioned pulp surface was noted only with CV. This
was because the strong alkali caused necrotic layers
and dentin–like hard tissue underneath the sectioned
pulp surface were formed, as previously reported.
Although dentin–like hard tissue underneath the
sectioned pulp surface are formed within a short
period by new pulp capping agents, there have been
reports regarding their mechanism that neupep-
tides and gene products in the dental pulp acted
with the differentiation of odontoblasts, and the
formation of restorative dentin was facilitated (1).
However, HAP used in our study showed no dentin–
like hard tissue formation underneath the sectioned
pulp surface. It has been reported regarding this
result that pulp capping agents less harmful to tissue
form no necrotic layers underneath the pulp capped
surface, and are not useful for dentin–like hard tissue
formation underneath the sectioned pulp surface (3).
Furthermore, it has also been reported that pulp
capping agents which did not form necrotic layers
induced restorative dentin, whereas they did not
easily facilitate local dentin–like hard tissue forma-
tion (1) underneath the sectioned pulp surface.

HAP in this study showed neither remarkable
abscess formation nor restorative dentin. This result
differed from those using other new pulp capping
agents. Although dentin–like hard tissue underneath
the sectioned pulp surface can prevent external irri-
tation and infections caused by micro–leakage, the
formation of strong dentin bridges is not always necessary because of the improvement in the adhesive ability of recent restorations (13), and the development of less harmful materials is desirable. Regarding pulpack V®, it was revealed that the influence of the toxicity of FC on the pulp tissue was strong, judging from the progress of necrotic layers confirmed between 3 days and 1 week after surgery.

Regarding dentin-like hard tissue that was not underneath the sectioned surface but on the pulpal wall, Kimura et al. (3) reported that increases in dentin-like hard tissue were noted in all the same pulp capping agents as we used in this study. This finding was similar to that when calcium hydroxide and hydroxyapatite granular agents were used.

In this study, micro CT findings confirmed the formation of dentin-like hard tissue on the pulpal wall in teeth other than those in which pulpotomy was performed. This suggested that the formation of dentin-like hard tissue on the pulpal wall was induced with age in rats.

However, the amount of dentin-like hard tissue formation was smaller with HAP than with calcivital. This difference may be related to differences in the elution of pulp capping agents into the pulp tissue, and further evaluation is necessary.

Considering the pH in pulp capping agents, and micro CT and patho-histological findings, it was confirmed that FC is highly toxic to the pulp tissue, and CV strongly irritates the pulp tissue from the perspective of necrotic layer formation. On the other hand, it was revealed that although the ability to induce hard tissue underneath pulp capping agents was weaker with sintered HAP than with CV, no inflammatory images were noted in the pulp after pulpotomy in HAP, and the sectioned surface recovered to a good condition at an early stage. Therefore, our results suggested that sintered HAP can be a substitute for CV and pulpack V®, as a pulp capping agent with reduced pulp irritation.

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