Reactions of the Dentin-Pulp Complex to Calcium Hydroxide Paste in Rats

Yuichiro Nishikawa1,2, Saeka Matsuda1,2, Yoshikazu Nakayasu1,2, Jin Toriya2, Yukiko Yokoi1,2, Masahito Shoumura1,2, Norimasa Okafuji3, Toshiyuki Kawakami3 and Naoto Osuga1,2

1) Department of Oral Health Promotion, Matsumoto Dental University Graduate School of Oral Medicine, Shiojiri, Japan
2) Department of Pediatric Dentistry, Matsumoto Dental University School of Dentistry, Shiojiri, Japan
3) Department of Hard Tissue Research, Matsumoto Dental University Graduate School of Oral Medicine, Shiojiri, Japan

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Abstract: In this study, we performed animal experiment using calcium hydroxide paste, a root canal filling material (Vitapex®, Neo Dental Chemical Products Co., Tokyo), on dentin-pulp complex and observed several tissue reactions. While rats were under general anesthesia, pulp exposure was done by drilling a cavity on the occlusal part of the maxillary molar using 1/2 round bur. Thereafter, calcium hydroxide paste was injected into the cavity, temporarily sealed with composite resin and photographed using m. CT. After 4 weeks, the experimental part was surgically excised as a whole and examined histologically. A thick tertiary dentin was formed in the area where idoform calcium hydroxide paste was directly applied. The newly formed hard tissue is composed of extremely irregular dentinal tubules. Although only few samples confirmed the formation of the so-called ‘dentin bridge’, the hard tissue could not be absolutely classified as a ‘reparative dentin’ but it can be recognized as a hard tissue connecting the dentin walls as it filled or covered the exposed pulp. The tertiary dentin formed underneath was thick. No necrotic layer was observed. Photographs of m. CT of the experimental side showed that the hard tissue formed in the root canal was radiopaque. However in the part of root canal where no hard tissue was formed, a radiolucent image was observed in the center. In this regard, a thick tertiary dentin was formed rather than a dentin bridge filling the gap. Since this is uncommon for calcium hydroxide, it is regarded as a distinct characteristic of Vitapex when applied to an exposed pulp. This phenomenon was thought to be due to the silicone oil component. The results suggest that the silicone oil reduced the alkalinity of the material thereby making it more amiable as a pulp capping agent. Furthermore, it is believed that the odontoblasts were promptly activated which led to the formation of large amounts of dentin.

Key words: Dentin-pulp complex, Calcium hydroxide paste, Reactions, Histopathology

Introduction

In Clinical Dentistry especially in Pediatric Dentistry, pulpotomy is a method of repairing infected coronal pulp to maintain the vitality of the remaining active dental pulp. The procedure involved the use of calcium hydroxide mixed with purified water and applied directly to the amputated pulp. This concept has been done for a long period of time with the objective of forming ‘dentin bridge’. Likewise, studies have been carried out many years ago1-4. Most researches were conducted using experimental animals such as dogs and observed the radiographic and histological changes5-9. In one of the studies, calcium hydroxide was applied on the amputated pulp and prompted the formation of necrotic layer. Dental pulp cells in the area also differentiated into odontoblasts and formed dentin. However, the dentin formed was extremely irregular and different from the physiologic histological structure10. Dentin can be classified as primary, secondary and tertiary depending on the time of its formation. Primary dentin is formed before tooth eruption; it is very regular and well-organized histologically. Secondary dentin is formed after completion of root and continues to form after tooth eruption. The boundary between primary and secondary dentin is distinct due to the presence of remodeling lines which appear darkly stained with hematoxylin in tissue preparation. Tertiary dentin is formed in response to pathological stimulation such as loss of dentin beyond normal enamel wear, caries, acid attack, abrasion, etc. Depending on the cause of its formation and the pathological tissue structure, it is also called as reparative or sclerotic dentin.

Several studies tested other materials aside from calcium hydroxide. However, dentin bridge formation on amputated surface is not as favorable compared to calcium hydroxide. Accordingly, researches have been proposed to improve the method and increase the success rate. Studies on other products have also been carried out to form dentin bridge artificially11,12.

In this study, a calcium hydroxide paste (Vitapex, Neo Dental
Chemica Products Co., Tokyo), a typical root canal filling material was applied on dentin-pulp complex in experimental animals. We obtained interesting information somewhat different from the results previously mentioned.

### Materials and Methods

#### Materials

Calcium hydroxide paste (Vitapex, Neo Dental Chemica Products Co., Tokyo) was used as a pulp applying material. The components of this product are shown in Table 1.

#### Laboratory animals

Male, 8 weeks old Wister rats were purchased from SLC Shizuoka and 20 healthy ones were used in the experiment after 2 weeks observation. The experimental animals were carefully kept in a temperature-controlled room with 12 hours of day/night shift for less stress and pain. The animals were also fed with sufficient food and water throughout the experiment. This study was approved by Matsumoto Dental University Animal Experiment Committee (approval number: #212 continuation - 2).

#### Experimental method

The animals were placed under general anesthesia by injecting sodium pentobarbital (Somnipentyl®) into the peritoneal cavity followed by taking photographs with m_CT. The rat was fixed to

### Table 1. Components of Vitapex

<table>
<thead>
<tr>
<th>Components</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium hydroxide</td>
<td>30.3</td>
</tr>
<tr>
<td>Iodoform</td>
<td>40.4</td>
</tr>
<tr>
<td>Silicone oil</td>
<td>22.4</td>
</tr>
<tr>
<td>Others</td>
<td>6.9</td>
</tr>
</tbody>
</table>

### Table 2. Result of outline evaluation by m_CT

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of teeth (general profile) where radiopacity could be confirmed</td>
<td>8</td>
</tr>
<tr>
<td>Number of teeth (imperfections) where radiopacity could not be confirmed</td>
<td>17</td>
</tr>
</tbody>
</table>

Figure 1. m_CT image. Case #19, immediately after surgery (a, b) and after 1 month (c, d)
Figure 2. m_CT image. Case #11, immediately after surgery (a, b) and after 1 month (c, d)
Figure 3. m_CT image. Case #17, immediately after surgery (a, b) and after 1 month (c, d)
Figure 4. m_CT image. Case #3, immediately after surgery (a, b) and after 1 month (c, d)
the plate, the left maxillary first molar was disinfected with tincture of iodine and an occlusal cavity was drilled using 1/2 round bur (manufactured by Merfar, Balliques) exposing the coronal pulp. Calcium hydroxide paste was then injected into cavity. Post-operative m_CT images were taken to confirm the status of treatment. After 4 weeks, the experimental part was removed as one block. The imaging conditions of m_CT were 70 kV for tube voltage and 120μA for tube current. Specimens were prepared for histological sections in a routine manner. Briefly, the specimens were fixed in 10% neutral buffered formalin solution and decalcified in 10% EDTA solution. The specimens were placed in ascending series of alcohol for dehydration and embedded in paraffin blocks. Serial sections of 4 microns were also prepared. Staining was carried out using hematoxylin-eosin (HE) and specimens were observed under an optical microscope. A total of 25 teeth were tested.

**Results**

*Examination by m_CT*

The results were confirmed using m_CT. Table 2 outlines the number of specimens where radiopaque image was confirmed.

Photographs taken using m_CT immediately after operation and one month after were compared. Immediately after operation, highly radiopaque images representing the implanted paste were
confirmed to be irregular in shape (Figs. 1-a, b, 2-a, b, 3-a, b, 4-a, b).

After one month, all radiopaque images decreased in size (Figs. 1-c,d, 2-c,d, 3-c,d, 4-c,d). Furthermore, m_CT image of one sample (Fig 1-d) showed collapse of the radiopaque image. In most cases, the implanted paste was observed to be in contact with the pulp or to the root canal wall. There was an increased in the width of the root canal compared to the pre-operative images (Figs. 1-d, 2-d, 3-d, 4-d). Some radiopaque images were considered to be due to narrowing of the root canal. This was specifically confirmed in one of the specimens (Fig. 3-d).

**Histopathological examination**

The radiopacities observed in m_CT images which were regarded as the formation of dentin on the dental pulp were further investigated by histological examination.

Histologically, the m_CT images in Fig. 1 showed conspicuous formation of tertiary dentin (Fig. 5-a). The formation of tertiary dentin occluded or covered the root canal beneath the crown (Fig. 5-b). In another part of root canal of the same tooth, irregular dentin formation was noted on both sides of the root canal specifically at the boundary of the root and crown of the tooth resulting to the occlusion of the canal (Fig. 5-c).
Photographs from m_CT in Fig. 2 showed radiopacities in three root canals. The formation of tertiary dentin was confirmed histopathologically considered to be due to the injected paste (Fig. 6-a). The tertiary dentin formed was thick and the staining was different from the surrounding tissues. The dentinal tubules were also unclear and irregular. Moreover, the newly formed dentin occluded the root canal opening (Fig. 6-b). The dentin in this part was also very peculiar. The capillaries in the area were highly congested. In some cases, bleeding can be observed. There were practically odontoblasts in the dental pulp of the root canal (Fig. 6-c).

Fig. 7-a shows the histological section of the specimen showed in Fig. 3. Although hyperemia of the pulp was observed, the arrangement of physiological odontoblasts was recognized and it was obvious that the pulp was active (Fig. 7-b). On the other hand, an extremely thick irregular dentin was formed in the pulpal side mostly occluding the lumen of the root canal (Fig. 7-c).

Fig. 8-a shows the histological image of the specimen shown in Fig 4. Many small vacuoles were observed in the pulp cavity (Fig. 8-b,c).

Discussion

The present study examined the subsequent tissue reactions in dentin-pulp complex following the direct application of Vitapex®. As previously mentioned, Vitapex is a root canal filling material dispense in a syringe and mainly consists of calcium hydroxide. It became popular since it is convenient to use (no kneading required) and offers favorable results. Moreover, basic researches have proven the capability of calcium hydroxide to form dentin bridge when used as a pulp capping material.

Calcium hydroxide is mainly used for dental pulp capping. This is because calcium hydroxide was thought to induce hard tissue formation better than other materials. Variations in the period of attachment of calcium hydroxide were observed histologically in rat molar tooth pulp and it was mentioned that hard tissue rescaling of the exposed pulp (dentin bridge) will be formed after 2 weeks in most cases. Nevertheless in another study, hard tissue formation (dentin bridge) induced by calcium hydroxide occurred after long term observation.

In a clinical experimental research by Fujisaki et al. (1995), they reported the course of observation after pulpotomy of decayed teeth with calcium hydroxide in pediatric out-patient using radiograph and suggested the need for periodic radiographic follow-up.

Changes in the width of pulp cavity was evaluated using different pulp capping agents as well as CO2 laser to know their effects on hard tissue formation through m_CT and histological sections. The pulp capping agents were partially observed and hard tissue formation was detected underneath the materials.

It is evident that calcium hydroxide can induce hard tissue formation when applied on pulp tissue. In some research showed that calcification occurred in cellular organelles such as in mitochondria and that cell residues were present in the necrotic layer formed by calcium hydroxide after pulp capping. This was shown by von Kossa positive granules indicating that calcification took place. Consequently, the hard tissue is said to be “heterotrophic calcification”.

Furthermore, analysis using electron microscope showed that the initial calcification started just below the necrotic layer and it was initiated by cell death.

In order to improve handling, several reports investigated the use of calcium hydroxide with eugenol and polyethylene glycol for pulp capping. However, histological sections did not show hard tissue formation either after short term (3 days) or long term (7 days) observation. Although the study considered 7 days as long term, this is not considered as long term observation.

Although many articles showed relatively positive results on hard tissue formation (dentin formation) by calcium hydroxide, other reports also mentioned that one of the harmful effects is the formation of necrotic layer. This is due to the high pH of calcium hydroxide causing the formation of rapid necrotic layer and immobilization of the pulp tissue (blood vessels, nerves etc.). The possibility of forming remnants of small pores and cracks during subsequent calcification was also mentioned.

The ability of calcium hydroxide was further investigated by using it in artificial bone defect. The transition of radioactive calcium in the body fluid to the newly hardened tissue after pulp treatment was also explored. The tracer showed that calcium from calcium hydroxide was transferred into the body fluids and was utilized to form hard tissues.

Aside from calcium hydroxide, calcium carbonate was used as dental pulp capping material. The results of m_CT and histopathology showed the formation of new dentin-like hard tissue just beneath the material. A similar study, hard tissue formation was evident histologically after using calcium hydroxide on exposed pulp sealed with composite resin. Comparison among formocresol, calcium hydroxide and polymeric hyaluronic acid to repair dentin after viable pulp amputation was also carried out. No distinct dentin formation occurred in polymeric hyaluronic acid formulation but the formocresol and calcium hydroxide showed excellent effect on the formation of dentin bridge suggesting that those materials are excellent in the formation of restored dentin. Based on the results of previous researches, the knowledge and future prospects of aiming a treatment for the regeneration of dentin-pulp complex have been recently described.

There are so many available root canal filling materials in the market for clinical use. Among those, the most representative is the calcium hydroxide paste (Vitapex), used in this study to show the reaction of dentin-pulp complex. As shown in Table 1, the three main components of this product are calcium hydroxide,
iodoform and silicone oil\textsuperscript{13}. The advantage of this product is that it is ready to use. It is already kneaded with silicone oil and there is no need to mix calcium hydroxide and iodoform in water. It is well known that calcium hydroxide exhibits strong alkalinity and it becomes necrotic when it comes in contact with living tissue. It has been known that necrotic layer is formed when the material is applied to the amputated portion of the pulp\textsuperscript{2,3}. However, it was proven by many animal experiments that the injurious action seemed to play a role in forming dentin bridge\textsuperscript{4-9}.

In the present study, due to the large stenosis of the root canal, the radiopaque image was not possibly confirmed in 17/25 specimens in m_CT. This is thought to be mainly caused by the infection of the dental pulp due to collapse of resin during the course of the experiment. Moreover, the success rate in forming dentin bridge is not high which may indicate that Vitapex is not an appropriate pulp capping agent. However, the results of histopathological examination based on m_CT images showing hard tissue formation is remarkable. Results showed the formation of irregular dentin. First, structures that were considered as paste materials or residues were observed. Second, remarkable necrosis was hardly observed in the vital dental pulp tissue. This material is kneaded with silicone making it water insoluble so it did not directly damage the pulp tissue or create a necrotic tissue. Moreover, the alkalinity of the material was probably decreased by the incorporation of silicone oil.

Dentin bridge was confirmed in few samples. This is considered to be the effect of calcium hydroxide in Vitapex when applied to the pulp. Although that was the case, it was quite interesting that the formation of necrotic layer did not occur. The hard tissue that was formed is not a fine hard tissue that connects one side of the root canal to the other (dentin bridge) nevertheless, it filled the gap. Since the hard tissue filled the gap, the top part can still be recognized as a ‘bridge’ with the thick tertiary dentin formed underneath. Because this is not a general style of calcium hydroxide to form dentin bridge, it was considered to be a characteristic phenomenon of Vitapex due to the incorporation silicone oil.

An extremely large amount of irregular dentin formation was formed on the dentin wall. The samples in which m_CT showed radiopaque images showed that dentin was formed in the root canal which would mean stenosis of the root canal. In addition, some were even closed or obliterated. However, in the central part of the root canal where no dentin was formed, a radiolucent image was confirmed. This is probably due to the gentle alkaline action of the material due to silicone oil. Moreover, the odontoblasts were activated to rapidly form large amounts of dentin.

Most of the newly formed hard tissues have unclear tubules. There were cells in some areas which were trapped in the hard tissue. Although undifferentiated mesenchymal cells differentiated into odontoblasts, the differentiation is not perfect because they formed bone-like dentin having irregular structure and encapsulated cells.

The hard tissue (bone-like dentin) formed this time did not meet the criteria to be clearly considered as a dentin bridge in the dental pulp. Many of them had constricted structure due to the active increase in dentin in the experimental part.

Regarding the small vacuole-like structures in the pulp, these were probably derived from the silicone oil which is the basic component of Vitapex. The same description was mentioned in a study where a similar material was implanted in the subcutaneous tissue\textsuperscript{10}. In addition, irregular bone-like dentin was formed due to the formation of large amount of dentin in a very short period of time.

In conclusion, formation of bone-like dentin was mostly observed in this experiment. The newly formed hard tissue does not have definite tubular structure. Although undifferentiated mesenchymal cells of the pulp differentiated into odontoblasts, the differentiation was not proper and they only formed bone-like dentin, having irregular structure. The cells were also found to be encapsulated. It is believed that the huge amount of irregular bone-like dentin was rapidly formed in a short period of time.

**Acknowledgments**

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**Conflict of Interest**

The authors have declared that no conflict of interest.

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