

## Research Note

# Histochemical Characteristics of Tertiary Dentin Due to Calcium Hydroxide Paste in Rats

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**Abstract:** Calcium hydroxide is mainly used for dental pulp capping and it is thought that it induces hard tissue formation far better than other materials. Experimentally verifying this fact, Nishikawa et al revealed that bone-like dentin corresponding to tertiary dentin is rapidly formed when calcium hydroxide is applied directly to the pulp. Utilizing the same experimental system, histochemical study of the newly formed hard tissue (reparative dentin) was carried out and the results thereof were reported. Thick and irregular reparative dentin was formed in the pulp cavity and partial narrowing of the root canal was observed in m-CT. Histopathologically, the irregular reparative dentin increased its thickness obliterating the root canal having a different Azan staining of aniline blue compared to primary dentin. Numerous cellular inclusion bodies were also trapped inside the thick dentin. Furthermore, with Schmorl's thionine picric acid staining, thick reparative dentin was noted around the pulp cavity and dentin. The dentin was densely stained with picric acid with different staining ability from the surrounding dentin. In addition, it was clearly confirmed that many cells were trapped in reparative dentin. The results of the experiment suggest that the characteristics of the newly formed reparative dentin is comparable to tertiary dentin.

**Key words:** Tertiary dentin, Reparative dentin, Calcium hydroxide paste

### Introduction

Treatment of dental pulp in deciduous teeth includes pulp sedation, pulpotomy, pulpectomy and tooth extraction. The material mainly used in pulpotomy is calcium hydroxide which is mixed with purified water and applied directly to the amputated surface of the pulp forming the so-called 'dentin bridge'. It was shown that calcium hydroxide induces superior hard tissue formation compared to other materials<sup>1-6)</sup>.

Nishikawa et al.<sup>7)</sup> conducted fundamental experiments on pulpotomy using rats and found out that bone-like dentin corresponding to tertiary dentin rapidly formed when calcium hydroxide was applied directly to the pulp. Therefore, in this same experiment, we added a few histochemical studies on the structure of the hard tissue (bone-like dentin) that was formed using the same experimental system and the results thereof are discussed.

### Materials and Methods

The experimental system used is similar to Nishikawa et al.<sup>7)</sup>. Iodoform hydrous oxide calcium hydroxide paste (Vitapex<sup>®</sup>, Neo Dental Chemical Product Co., Tokyo) was used for the root canal material and Wister rats (male and female, 8 weeks old, SLC, Shizuoka) were used as experimental animals. One-half round bur (Melfer and Torx) was used to access the pulp chamber. Briefly, the animals were placed under general anesthesia with pentobarbital sodium (Somnopenyl<sup>®</sup>) in the abdominal cavity and the crown of the first molar was perforated to expose the pulp. After pulp exposure, calcium hydroxide paste was injected in the same area. Four weeks later, the first molar as well as the surrounding tissues were removed as a block. The tissues were fixed

in 10% neutral buffered formalin solution, decalcified in 10% EDTA solution and dehydrated in increasing series of alcohol. Then after, the tissues were embedded in paraffin and serial sections of 4 microns were prepared following the routine method. Besides hematoxylin-eosin (HE) staining, the sections were also stained with thionin picric acid staining of Schmorl and Azan. This study was conducted with the approval of Matsumoto Dental University Animal Experiment Screening Committee.

### Results

In the m-CT photograph, an increased in the width of the dental pulp tissue of the root canal in contact with the implanted material was clearly visible as a radiopaque image compared to the photograph immediately taken after the operation (Fig.1-a). In some samples, the root canals had not closed. Some of the radiopaque images were considered to be stenosis or narrowing of the root canal which is strongly observed in the upper part of the root canal. Below the radiopacity, radiolucency of the root canal was confirmed (Fig. 1-b).

HE staining shows that calcium hydroxide was directly applied to the pulp filling the gap. The newly formed dentin is thick with highly irregular dentinal tubules. The newly formed hard tissue is called reparative dentin which filled the root canal gap or space. Formation of reparative dentin that filled the root canal part was remarkable which is very different from the original dentin. The difference is not only in the arrangement or structure of dentinal tubules but also in the staining ability. The reparative dentin stained differently from the surrounding hard tissues and the dentinal tubules were also ambiguous and irregular (Fig. 2-a, b).

Azan staining also showed increased formation of thick reparative dentin around the pulp cavity similar to that observed in HE staining.

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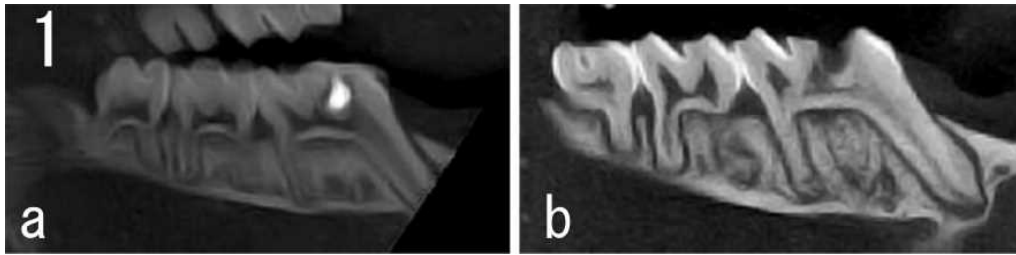


Figure 1. m\_CT image. immediately after surgery (a) and after 1 month (b)

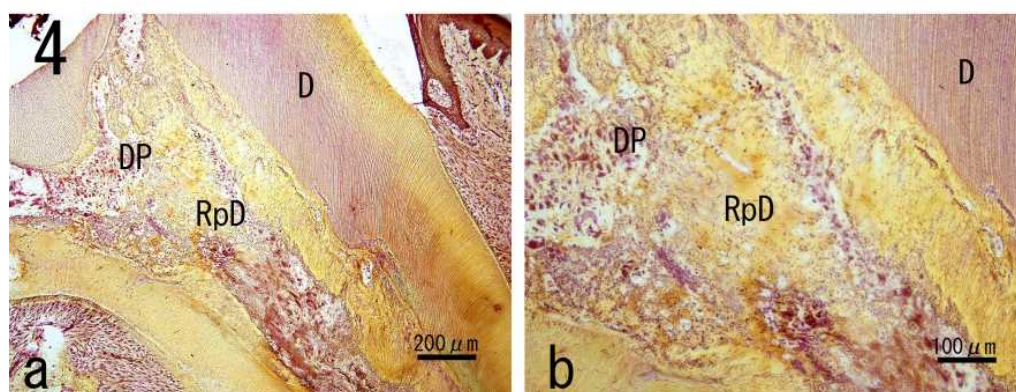
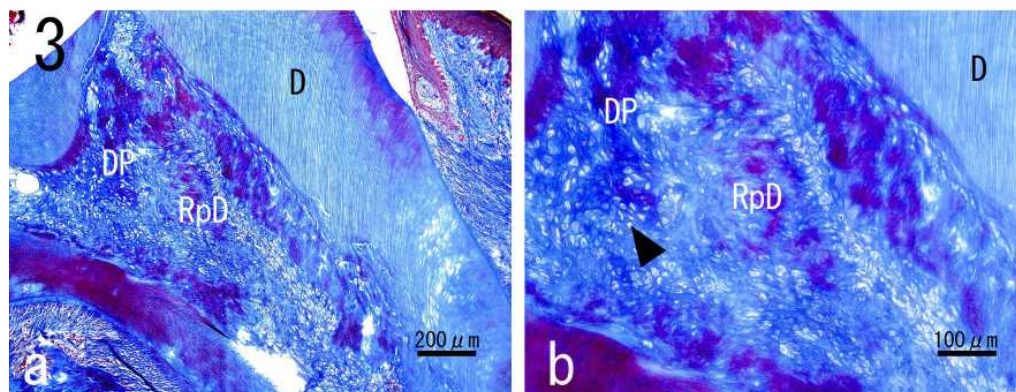
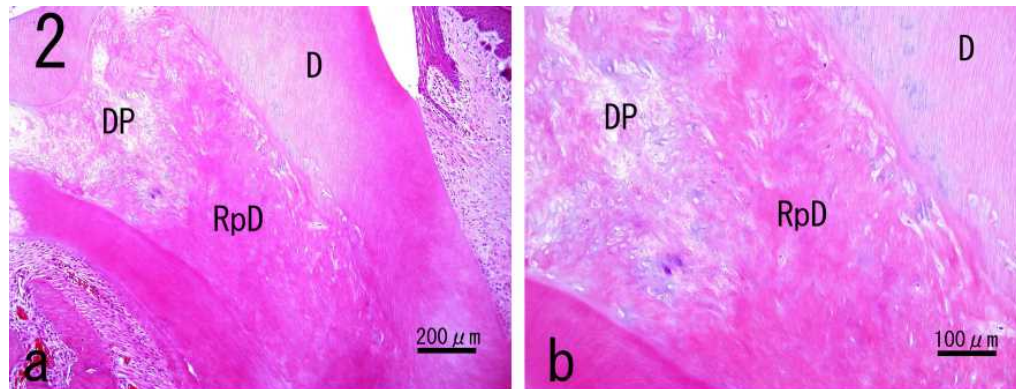


Figure 2. Hematoxylin and eosin staining. after 1 month, low power magnification (a), high power magnification (b).

Figure 3. Azan staining. after 1 month, low power magnification (a), high power magnification (b). Numerous cellular inclusion bodies were confirmed inside the newly formed dentin (arrowhead).

Figure 4. Thionin picric acid staining of Schmorl. after 1 month, low power magnification (a), high power magnification (b). D:dentin ; DP : dental pulp ; RpD : reparative dentin.

The root canal was almost occluded. Unlike the primary dentin, the staining ability of reparative dentin in aniline blue is different. Numerous cellular inclusion bodies were confirmed inside the newly formed dentin. The dentinal tubules in newly formed dentin were markedly ambiguous than in the original dentin (Fig. 3-a, b).

Schmorl's thionine picric acid staining also showed the newly formed reparative dentin around the pulp cavity similar to those observed in HE and Azan stainings. The dentin formed was densely stained with picric acid and the staining ability is different from the surrounding dentin. Also, there were many cellular inclusion bodies not stained in the nucleus in the reparative dentin. The dentinal tubules were unclear compared to those of the surrounding dentin and the arrangement of the odontoblasts was also irregular. Stenosis of the root canal in contact with the implanted paste was evident caused by the vigorously formed reparative dentin (Fig 4-a, b).

### Discussion

Tertiary dentin is classified as reparative dentin formed by the odontoblasts. The differentiated odontoblast-like cells are produced as a reaction to various stimuli such as abrasion, caries and restorations. Formation is made only by directly stimulating the odontoblasts and its structures and the quantity depends on the strength and duration of stimulation. Compared to secondary dentin, the tubules are irregular or none at all. The cells forming the tertiary dentin may be arranged in a row on the dentin surface or may be embedded in the dentin<sup>8)</sup>.

As mentioned by Nishikawa *et al.*<sup>7)</sup>, calcium hydroxide (Vitapex, Neo Reagent Industry Co., Ltd., Tokyo) is a typical root canal filling material used in root canal treatment. An experiment in rats was conducted and the tissue reaction in dentin/pulp complex was treated with calcium hydroxide. The mechanism by which calcium hydroxide induces hard tissue formation in exposed viable pulp after pulpotomy is due to the presence of cell debris present in the necrotic layer or immediately below it. It is clear that calcification occurred as shown by the appearance of Von kossa positive granules. Therefore, this calcification (hard tissue formation) is said to be a heterotrophic calcification<sup>9)</sup>. Furthermore, based on the analysis of the results using electron microscope, it has been shown that the initial calcification on the amputated dental pulp where calcium hydroxide was applied, started just below the necrotic layer and the calcification was initiated by cell death<sup>10)</sup>. In case of an extremely high pH material such as pure calcium hydroxide, the structure of the pulp tissue (blood vessels, nerves, etc) was preserved in spite of the rapidly progressing formation of necrotic layer. The tissue components remained during the subsequent progression of calcification but the possibility of forming small pores and cracks was also mentioned<sup>11)</sup>.

At this time, we will focus on histochemical findings of the newly formed hard tissues (reparative dentin). First, at the perforation site, structures that were considered to be paste material or residues thereof were observed. However, thick reparative dentin was also observed without significant necrosis in the dental pulp tissue in contact with it. This is probably because the paste is kneaded with silicone oil and does not directly damage the tissue. The strong alkalinity of calcium hydroxide has been eliminated and so necrotic tissue was not formed. In addition, it was not a neat dentin bridge that was formed but something similar to filling the gap between the walls of the root canal. Nevertheless, it was still considered as a dentin bridge because of its continuity and the newly formed reparative dentin was thick. This dentin bridge formation is not typical for calcium hydroxide and is considered to be the characteristic of Vitapex due to the incorporation of silicone oil<sup>17)</sup>. This hard tissue showed a distinct staining difference

compared to the normal dentin in HE, Azan and Schmorl's thionine picric acid stainings. In reparative dentin, the structure of the tubules as well as the arrangement of odontoblasts are unclear. In Azan staining, many cellular inclusion bodies in reparative dentin have grown in the pulp cavity. This is due to the decrease in the alkaline potency of Vitapex by silicone oil. The activation of odontoblasts and newly differentiated odontoblast-like cells rapidly formed a large amount of dentin. Therefore, the differentiation between odontoblasts from undifferentiated mesenchymal cells of the pulp and the odontoblast-like cells that formed reparative dentin could not be properly discerned. However, the newly formed reparative dentin has irregular structure and the cells were trapped inside. This is thought to be due to the rapid and large dentin formation in an extremely short period of time specifically one month in this experiment<sup>7)</sup>. In that sense, the reparative dentin formed in this experiment was considered to be classified as tertiary dentin.

For future studies, we will also consider whether odontoblasts forming the reparative (tertiary) dentin can be activated by normal cells in the area or from other sites, etc.

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### Conflict of Interest

The authors have declared that no conflict of interest.

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