Original Article

Micro-computerized tomographic Evaluation of Reparative Dentin Formation after Direct Pulp Capping *In vivo*

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Abstract

Dental pulp vitality can be preserved after direct pulp capping with materials that can promote hard tissue formation. The comparison of efficacy regarding dentin bridge formation of different direct pulp capping materials is still limited. The objective of this study was to analyze and quantify the reparative dentin formation after direct pulp capping with different direct pulp capping materials on mechanically injured dental pulp of rat molars through micro-computerized tomographic analysis. Fifteen rat molars were mechanically exposed and assigned into three groups according to the direct pulp capping materials used: no treatment (control), calcium hydroxide (Dycal®) and Biodentine. After four weeks of treatment, the teeth were collected and processed for micro-computerized tomographic imaging and histological evaluation. The ratio between total dentin volume and tissue volume was evaluated using micro-computerized tomographic analysis software. Hematoxylin and eosin staining of the samples treated with calcium hydroxide and Biodentine revealed the hard tissue formation resembled reparative dentin around the exposure site where the capping materials were placed. Micro-computerized tomographic imaging identified the location of reparative dentin formation after direct pulp capping. The average ratio of total dentin volume and tissue volume was 0.6055±0.0641 in the control group, 0.7381±0.0535 in the Biodentine group and 0.7099±0.0361 in the calcium hydroxide group. The calcium hydroxide and Biodentine® treated groups had a significantly higher total of dentin volume and tissue volume ratio compared with the untreated control group (p=0.008). However, no statistical significance was observed between the calcium hydroxide and the Biodentine® treated groups. Calcium hydroxide and Biodentine® can promote reparative dentin formation when used as direct pulp capping materials. In addition, micro-computerized tomographic imaging can be considered as a standard technique to quantify and localize the location of reparative dentin formation.

Keywords: Biodentine, Direct pulp capping, Reparative dentin, Micro-CT, Vital pulp therapy

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Introduction

Vital pulp therapy is a treatment to preserve the tooth vitality of teeth destroyed by dental caries, trauma, or restorative procedures. This technique is important especially for young permanent teeth with incomplete root development.[1] Direct pulp capping is one of the vital pulp therapy techniques. It is employed for pinpoint mechanical exposure of the dental pulp.[2] The capping material is placed in contact with the exposed pulp tissue in order to promote pulp tissue healing and subsequently induces the formation of reparative dentin to prevent bacterial invasion and to preserve dental pulp vitality.[3]

There are many materials that are used for direct pulp capping. Calcium hydroxide[4-6] is generally used as pulp capping material however Biodentine has recently been introduced as the candidate material of choice.[7] Clinical studies have shown several aspects of success with these materials such as the absence of symptoms or pathological results.[8] In both human and animal studies, histological evaluation of pulp capping materials assessed the inflammatory response of pulp and revealed that dentin bridge formation was stimulated underneath the material.[5,7,9]

Although the histologic evaluation of reparative dentin formation after direct pulp capping is commonly accepted as the gold standard, micro-computerized tomographic imaging is a noninvasive technique that can preserve dental hard tissue after investigation.[10] Hence, this study aimed to analyze and quantify the reparative dentin formation after direct pulp capping on mechanically injured dental pulp of rat molars through micro-computerized tomographic analysis.

Materials and Methods

Rat Molar Mechanical Pulp Injury Model

The animal experiment was approved by the Ethical Committee of the Faculty of Dentistry, Chulalongkorn University (Ethic NO.1732001). Eight 8-week-old, male Wistar albino rats *(Rattus norvegicus)* were obtained from the National Laboratory Animal Center, Mahidol University. Rats were given general anesthesia with sodium pentobarbital (40 mg/kg body weight) by intraperitoneal injection. Then, local anesthesia was applied at the surgical site with 2 % lidocaine with epinephrine 1:100,000 (4.4 mg/kg/dose). Intentional mechanical injury was performed by drilling on the mesial surface of first maxillary molars until near exposure, followed by creating a mechanical exposure with a sharp instrument and treated with three different pulp capping agents implanted into the exposure site using a blunt steel probe. The teeth were assigned into three groups: no treatment (control), calcium hydroxide (Dycal®, Dentsply, USA) or Biodentine (Septodont, France). Thereafter, the cavities were sealed with glass ionomer restorative materials (Fuji II LC®, GC, Tokyo). Four weeks after surgery, the animals were euthanized by sodium pentobarbital via intraperitoneal (120 mg/kg body weight) delivery. The use of sodium pentobarbital was modeled from the study of Limjeerajarus *et al*[11], and approved from the Ethical Committee of the Faculty of Dentistry, Chulalongkorn University to be a non-suffering method for rats. The whole maxilla of rats was sectioned and fixed in 10 % buffered formalin for 48 hours.

Micro-computerized tomography analysis

The whole maxilla was separated to the right and the left side. Hard tissue formation was evaluated using micro-computerized tomography (μCT35, Scanco Medical, Switzerland). The specimens were scanned with the following parameters: 70 kVp, 114 μA, 8 watts, and voxel size 10 μm. The same threshold was assigned to all sample analyses in the study. The analyses were performed using software from Scanco Medical. Each sample was analyzed with 60 slides in horizontal plane that the exposure involved. The area of pulp was selected

by the inner wall of dentin (Fig 1h). The ratio of total dentin volume and tissue volume was evaluated.

Histological analysis

Specimens were decalcified and embedded in paraffin blocks. The sections of two microns in thickness were obtained and stained with hematoxylin & eosin for histological evaluation. Each sample was investigated for tissue reaction by a blind investigator. The location and severity of inflammatory reaction, the presence and quality of dentin bridge formation were evaluated.

Statistical analysis

Results of the micro-computerized tomography analysis were expressed as mean±standard deviation. The Kruskal-Wallis test was performed to determine significant difference. Then, the Mann-Whitney test was run between the control and the experimental groups. All data was analyzed to determine the significant difference between the samples using IBM SPSS software version 22.0 (IBM, Armonk, NY, USA). Results were considered statistically significant at $p<0.05$.

Results

Hard tissue formation occurred underneath pulp capping material four weeks after the pulp capping procedure. Micro-computerized tomographic imaging revealed hard tissue formation at the exposure site and the total dentin volume/tissue volume ratio at the level of the mechanical pulp exposure of the experimental groups were higher than those of the control group (Fig. 2). The average ratio of total dentin volume/tissue volume was 0.6055±0.0641 in the control group, 0.7381±0.0535 in the Biodentine treated group and 0.7099±0.0361 in the calcium hydroxide treated group. The calcium hydroxide and Biodentine treated groups exhibited significantly higher total dentin volume/tissue volume ratio than the untreated control ($p=0.008$). Moreover, total dentin volume/tissue volume ratio of the Biodentine treated group was higher than the calcium hydroxide group. However, there was no statistically significant difference ($p=0.421$). A three-dimensional model of the defects is also demonstrated in Figure 1g.

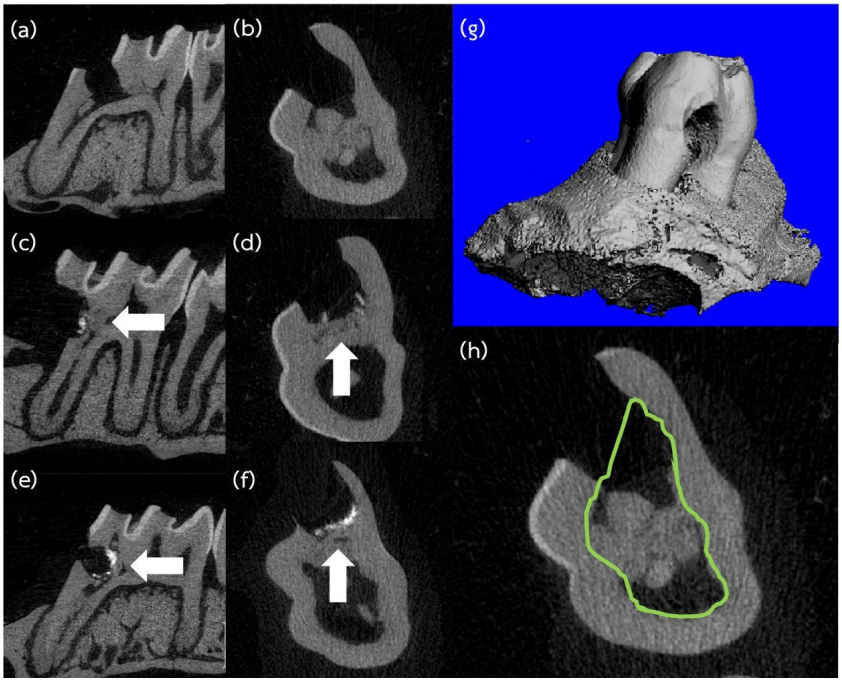

Figure 1 *Micro-computerized tomography images which show hard tissue formation occurring underneath pulp capping material after pulp capping procedures: the untreated control (a,b), calcium hydroxide (c,d), and Biodentine (e,f). Representative image of three-dimensional model of the defects (g). The area was selected to calculate the ratio of total dentin volume/tissue volume (h).*

Histological staining of reparative dentin formation showed the additional information of pulpal response after direct pulp capping (Fig. 3). The untreated group showed only fibrous tissue formation near the exposure site and abundant inflammatory response. Inflammatory cell infiltration involved more than 75 % of coronal pulp tissue. There was no reparative dentin formation in the control group. On the contrary, the calcium hydroxide and the Biodentine treated groups stimulated bridge formation with mineralized reparative dentin. Reparative dentin was seen at the exposure site and adjacent to former dentin close to the exposure site. Moreover, pulp tissue underneath the reparative dentin formation was similar to normal pulp tissue. There was minimal inflammation in the group treated with calcium hydroxide or Biodentine. Also, there were less blood vessel dilation and inflammatiry cell infiltration than in the control group.

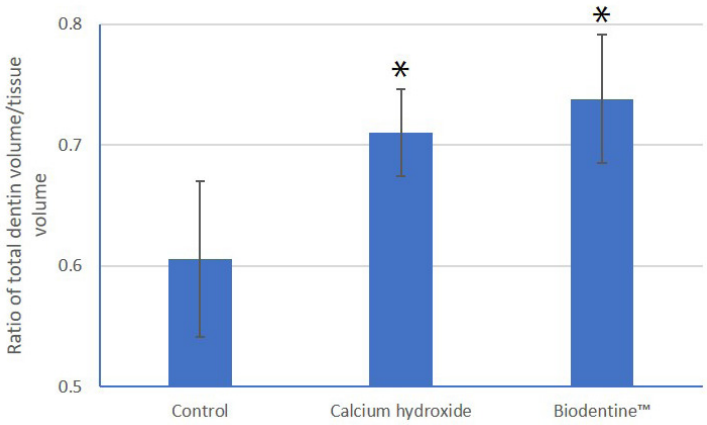

Figure 2 *Graph demonstrates the ratio of total dentin volume/tissue volume. The calcium hydroxide and Biodentine® treated group had a significantly higher ratio of total dentin volume/tissue volume than the control group. However, there was no significant difference between both pulp capping material treated groups. (* p<0.05).*

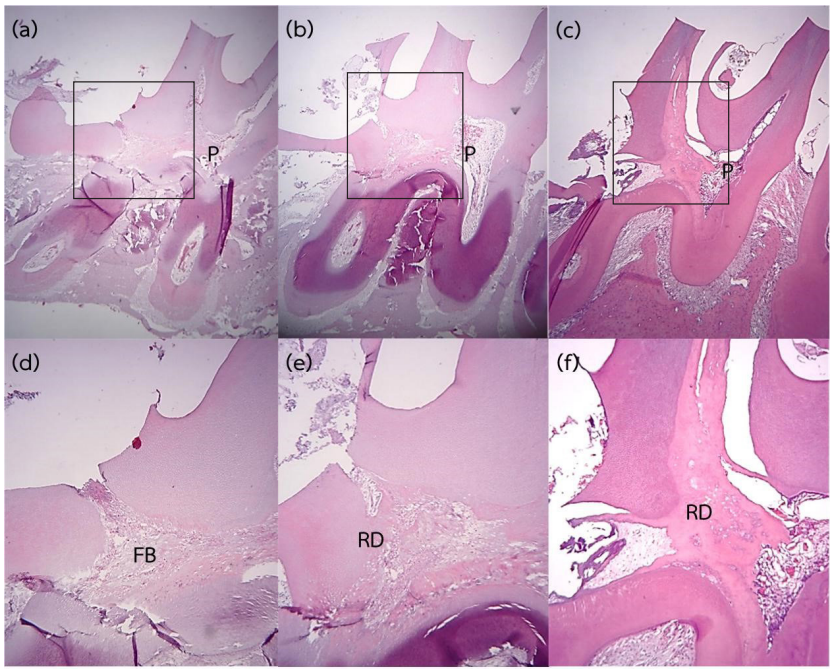

Figure 3 *Histological evaluation showed pulpal response after direct pulp capping: reparative dentin formation and inflammatory response (Magnification 4x (a-c) and 10x (d-e)). Reparative dentin formation (RD) occurred at exposure site and there was minimal inflammation after direct pulp capping with calcium hydroxide (b,e) or Biodentine (c,f). There were severe inflammation and reparation with fibrous tissue (FB) in the control group (a,d). (P; dental pulp).*

Discussion

Direct pulp capping is a treatment for vital pulp therapy. Pulp capping material should have proper properties for the healing process such as anti-bacterial, biocompatibility, hard tissue formation and tissue regeneration.[12-15] Presently, there are many materials used for this procedure. Calcium hydroxide paste or Dycal® and Biodentine are among the most popular material that dentists use in clinical practice. However, these materials are different in properties and results of treatment. It has been reported that both materials could promote dentin bridge formation after direct pulp capping.[13,16]

Micro-computerized tomography is one of the techniques that is employed to evaluate hard tissue formation without the destruction of the specimens. Ishimoto *et al* and Kim *et al* revealed that hard tissue formation was shown in micro-computerized tomography within four weeks after direct pulp capping.[10,17] Furthermore, the result of micro-computerized tomography was shown in three dimensions and the quantification of hard tissue was calculated with micro-computerized tomography software. In the present study, the total dentin volume/tissue volume ratio was analyzed based on the calculation of the volume of mineral tissue that is similar to hydroxyapatite and the total volume of interest. We selected the volume of interest that was involved in the mechanical exposure. It revealed hard tissue formation after mechanical exposure and the application of pulp capping materials in three dimensions. Hard tissue formation occurred after direct pulp capping. The use of biodentine and calcium hydroxide resulted in the formation of hard tissue with a significantly higher average of the total dentin volume/tissue volume ratio compared with the untreated group. However, the Biodentine group showed a higher average of the total dentin volume/tissue volume ratio than the calcium hydroxide group but there was no significant difference. According to Nowicka *et al,* the dentin bridge formation was evaluated with cone-beam computed tomographic (CBCT) imaging, the formation of bridge after direct pulp capping with calcium hydroxide had a similar result to Biodentine.[18]

While calcium hydroxide has antibacterial properties because of high pH, the result of calcium hydroxide is poor properties of dentin bridge formation. Calcium hydroxide can be resolved over time. Then, dentin bridge after direct pulp capping with calcium hydroxide revealed tunnel defect and osteodentin.[14,17,19-21] It consisted of porosity that can be a result of penetration of bacteria into the pulp cavity.[19] While, the dentin bridge formation of Biodentine had less porosity than calcium hydroxide.[17] Furthermore, the use of Biodentine showed a less inflammatory response in both the clinical[7] and animal studies.[22] Although many studies revealed that Biodentine stimulated reparative dentin formation and was a more preferable result than calcium hydroxide when used as pulp capping material. There were no previous studies done in the aspect of quantification by using micro-computerized tomography.

Although histological analysis is the most common investigation of reparative dentin formation after vital pulp therapy, it showed only two dimensions and could not showed the result in volume of reparative dentin formation. Moreover, it had technical sensitive because it required a specialist for interpretation. On the other hand, micro-computerized tomography showed the result in three dimensions and the specimens were not destroyed after investigation. The difference of former and reparative dentin could not be observed by using micro-computerized tomography. Therefore, histological staining was needed to confirm and identify reparative or former dentin.

Recently, the use of calcium hydroxide as pulp capping material has decreased. Despite being the gold standard for direct pulp capping in the past due to its antibacterial properties, calcium hydroxide exhibited

several drawbacks which can affect clinical outcomes. These disadvantages include pulpal inflammation and necrosis, microleakage from tunnel defects in the dentin bridge and degradation over the long term.[12,14] On the other hand, the dentin bridge completely formed after direct pulp capping with Biodentine and there was no inflammatory response in both the animal and clinical studies.[7,22]

Mechanism of reparative dentin formation after direct pulp capping occurred from the properties of the materials including high pH, antibacterial activity and calcium ion release.[23] Both calcium hydroxide and Biodentine have these properties. A high pH environment promoted osteogenic differentiation and bacterial irritation. Therefore, the process of reparative dentin formation was supposed to occur in this bacteria-free environment. Furthermore, calcium ions that were released from the materials was necessary for the formation of reparative dentin.

Mineral trioxide aggregate (MTA) is a pulp capping material. Because MTA is a calcium silicate based material in which the composition and properties is similar to Biodentine, many studies revealed favorable outcomes in both animal and clinical studies. From meta-analysis and a long-term study of MTA resulted in the decreased risk of failure and showed a significant higher success rate than calcium hydroxide.[24,25] High quality dentin bridge formation and less inflammation were observed after direct pulp capping with MTA.[26] Although calcium silicate base material revealed better results than the calcium hydroxide, MTA and Biodentine used in direct pulp capping procedures after caries removal in young permanent teeth showed a clinical outcome similar to calcium hydroxide.[27] Therefore, MTA is one of the suitable materials in vital pulp therapy. However, there has been no study done that studied the results of MTA using micro-computerized tomography. Therefore, micro-computerized tomographic evaluation of reparative dentin formation after direct pulp capping with MTA should be further studied.

Conclusion

Micro-computerized tomographic imaging can be considered as one of the standard techniques to quantify and localize the location of reparative dentin formation. Calcium hydroxide and Biodentine promote favorable results for direct pulp capping in terms of reparative dentin formation and reduction of inflammation.

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