Affected dentin remineralization after partial caries excavation (in vivo): the effect of iRoot® BP Plus application

N U Harahap¹, N Djauharie²*, and D Asrianti²

¹Conservative Dentistry Residency Program, Faculty of Dentistry, Universitas Indonesia, Jakarta, 10430, Indonesia
²Departement of Conservative Dentistry, Faculty of Dentistry, Universitas Indonesia, Jakarta, 10430, Indonesia

*E-mail: nila.setyopurnomo@gmail.com

Abstract. The removal of all infected dentin on deep carious lesions leads to a high risk of pulp exposure. Currently, minimally invasive treatment can be achieved by partially removing carious lesions. Partially removing the carious lesion and applying bioactive material, such as iRoot® BP Plus, can promote remineralization of the affected dentin. This study aimed to evaluate the remineralization of affected dentin by partially removing infected dentin or removing all infected dentin after the application of iRoot® BP Plus. Subjects were divided into two groups. In group I, infected dentin was partially removed, whereas in group II, all infected dentin was removed. Pixel grey value was measured in each group before and 4 weeks after the application of iRoot® BP Plus and then compared. Remineralization of the affected dentin occurred in both groups after the application of the material for 4 weeks.

1. Introduction

Dental caries result from ecological changes in biofilms, moving from balanced microorganism populations to acidogenic, aciduric, and cariogenic microorganism populations that develop due to continuous carbohydrate fermentation, decreasing the pH. This causes demineralization to be greater than remineralization, resulting in the loss of minerals in dental tissues [1,2]. Formerly, dental caries was considered an infectious disease; therefore, the treatment was the elimination of all bacteria [3]. The principles of cavity preparation according to GV Black served as guidelines for treating clinical restorations by removing all infected dentin until only healthy dentin remained [4]. The removal of caries in infected dentin frequently resulted in the opening of the pulp, causing inflammation of the pulp and necrosis of the tooth [5,6]. Research showed that it is not possible to remove all bacteria in a carious lesion [7]. Lacin found that 40% of bacteria persisted in some infected dentinal tubuli after a thorough excavation of deep carious lesions [8]. According to Bjørndal in 2008, deep carious lesions are defined as carious lesions that can be seen radiographically, spreading more than 70%–75% into dentin and limited by the radiopaque area adjacent to the pulp [9,10].

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After nearly a century, the concept of minimal intervention brought a change in the philosophy of care with the removal of all infected dentin, while affected dentin remained [1]. Selective caries tissue removal evolved because of changes in the understanding of the processes underlying new caries.
pathogenesis and advances in adhesive dental material sciences. Deep carious lesions in adult teeth can be treated with two approaches: stepwise excavation and one-step excavation. According to the International and American Associations for Dental Research in the 2016 International Caries Consensus Collaboration, the one-step excavation technique is called “selective removal to soft dentin,” which means caries removal by leaving some soft dentin [2,3].

Ricketts et al. evaluated the activity and development of restored lesions when infected dentin remained. They observed that one-step excavation selectively removes soft dentin; they also showed that the development of most lesions was impaired both clinically and radiographically and over time indicated the absence of microorganisms [11,12].

Advances in biomaterials, such as RM-GIC, MTA, Biodentin, Bioceramic, and others, allow the formation of dental bridges that can trigger healing [12,13]. These materials can release fluoride, calcium, and phosphate ions that promote precipitation or formation of apatite crystal over a partially demineralized collagen matrix [14]. According to Banerjee et al., the development of bioactive materials that trigger remineralization supports minimally invasive treatment in deep carious lesions, with partial caries removal [7].

Currently, a calcium silicate-based bioceramic material has been developed as pulp capping material, and one of the recognized brands is iRoot® BP Plus (Innovative Bioceramic Inc., Vancouver, Canada). This material is easy to use because it is available in ready-to-use packaging, does not shrink during hardening, is insoluble, is radiopaque, and is an aluminum-free calcium silicate-based material [15]. Tian et al. reported that at low pH, iRoot® BP Plus has a homogeneous superficial microstructure with thin, poreless crystal sheets, which has a nanoparticle component. The acidic environment disrupts the formation of apatite but still encourages the viability of MC3T3-E1 pre-osteoblast cells [15]. In a study conducted by Zhang et al. in 2015, iRoot® BP Plus was found to significantly stimulate healing of pulp tissue in mice. Therefore, further research using iRoot® BP Plus as a pulp capping material is highly recommended [16].

Therapy to improve remineralization can be evaluated by determining changes in the quality and quantity of minerals in the teeth. Therefore, clinicians need accurate diagnostic tools to monitor the remineralization of carious lesions. A very promising diagnostic tool that provides high-resolution images of tooth remineralization with a non-invasive technique is optical coherence tomography (OCT). The images of lesions before and after remineralization are compared in terms of relative mineral loss ΔZ (%vol × μm) obtained by high-resolution digital microcardiography and chemical changes obtained by infra-red spectroscopy [17]. However, this tool has a limited scanning area [18]. In addition, OCT equipment for dentistry is not yet available in Indonesia and is difficult to obtain.

Digital imaging also has the potential to improve diagnostic frequency and accuracy. Digora digital radiography can calculate the optical density of a pixel with sufficient degrees of sensitivity to detect small differences that cannot be seen by the human eye. Based on a study conducted by Nóbrega et al. in 2012, the digital indirect radiographic method, using Digora, was found to be an alternative method comparable to the direct method to measure the grey value of a biomaterial [19].

The remineralization of dentin with biomaterial applications has been investigated in vitro by various methods. In the early stages of remineralization, mineral foci are formed. The formation of mineral foci begins 2 days after the application of biomaterials. Observations after 14 and 28 days have indicated an increase in the number and shape of minerals [20]. In 2015, Prati et al. examined the biological perspectives of calcium silicate bioactive cement that was immersed in a simulated body fluid and analyzed using scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM/EDX). In the study, it was reported that the formation of spherulite minerals was visible after 7 days of bioactive material application. After 28 days, there was an increase in apatite, and the intensity increased over time [21]. Therefore, in the present study, it was assumed that there would also be an increase in the grey value by digital radiographs so that remineralization levels after 4 weeks could be calculated using digital imaging (Digora™ Optime).

The use of biomaterials triggers dentin remineralization. Dentin remineralization can occur as either classical or non-classical remineralization. In classical remineralization, crystal precipitation
only occurs when the dentin is in an environment containing many calcium and phosphate ions. Classical remineralization produces only extracellular remineralization, whereas in non-classical remineralization, biomimetic remineralization occurs so that intrafibrillar remineralization can occur. To date, there have been few reports of remineralized levels of affected dentin found during the disposal of some of the infected dentin while biomaterial was provided as pulp capping material. Therefore, the present research examined remineralization rates when some of the infected dentin was left closest to the pulp with iRoot® BP Plus application, as measured by Digora™ Optime digital radiography.

2. Methods
Research subjects were collected consecutively, over a 1-month period. After 1 month, subjects were divided into two groups, with five in each group for a total of 10 subjects.

The subjects were patients who visited the Teaching Hospital, Faculty of Dentistry, Universitas Indonesia, with complaints of premolar 1, premolar 2, molar 1, or molar 2 cavities without any spontaneous pain. Examination of tooth vitality was done with a cold thermal test (ethyl chloride). Teeth with site 1 and site 2 cavities or Black class 1 and 2 cavities were included. Subjects with deep carious lesions (D5) showed 0.25–1 mm dentin thickness of the pulp left on radiographic images. Subjects with carious lesion depth of 0.25–0.5 mm were included in Group I (infected dentin partially removed), and subjects with carious lesion depth of 0.6–1 mm were included in Group II (infected dentin entirely removed). After explanation of the study, patients provided signed informed consent.

In Group I, the tooth was isolated with a rubber dam, and dental caries detector dye (SableTM and Seek®, Ultradent, USA) was applied. After that, the dark green dyed part of the lesion nearest to the pulp was radiographically confirmed and partially excavated for superficial dentin caries (partially infected dentin) with a sterile excavator (EXC 63-64, Osung). The part of the wall and cavity base that was farther from the pulp was excavated for all infected dentin, leaving affected dentin. The tooth was cleaned and dried with cotton pellets. iRoot® BP Plus was applied up to 1-mm thick on the cavity base and confirmed with a ball-tipped periodontal probe (PUNC15, Osung). After that, a glass ionomer (GIC Fuji IX, GC Corp) was applied to cover the entire cavity. Patients were instructed to return within a period of 4 weeks.

In Group II, the tooth was isolated with a rubber dam, and dental caries detector dye was applied. After that, the dark green dyed area was excavated throughout the infected dentin with a sterile excavator until it turned light green. The tooth was cleaned and dried with cotton pellets. iRoot® BP Plus was applied up to 1-mm thick above the cavity base and confirmed with the ball-tipped periodontal probe. After that, a glass ionomer was applied to cover the entire cavity. Patients were instructed to return within a period of 4 weeks.

After 4 weeks of treatment, radiographic photographs were taken with the same initial angle. The pixel grey value of dentin was measured using Digora for Windows® (Soredex Orion Corporation, Helsinki, Finland). The calculation of the grey value was blinded and randomized, and the pixel grey values before and after treatment were compared.

The data obtained were analyzed statistically using SPSS ver. 20, using paired t-test in each group before and after treatment. Unpaired t-test was used to compare the results of both groups after treatment. When the data were not normal, Wilcoxon test was used.

3. Results
Based on the observation of all subjects, remineralization occurred in both groups. Before and after iRoot® BP Plus application, the group with the infected dentin partially removed as well as the group with infected dentin entirely removed showed positive changes.
Table 1. The average pixel grey value of dentin with infected dentin partially removed and infected dentin entirely removed before and after iRoot® BP Plus application

| Carious tissue removal                  | Pixel grey value of dentin |
|----------------------------------------|----------------------------|
|                                        | N         | Before treatment (Mean ± SD) | Four weeks after treatment (Mean ± SD) |
| Infected dentin partially removed      | 5         | 123.40 ± 8.691               | 151.40 ± 9.737                     |
| Infected dentin entirely removed       | 5         | 168.80 ± 17.541              | 195.40 ± 25.265                    |

Table 1 shows the values in both groups before and after iRoot® BP Plus application. Based on Shapiro–Wilk test, data distribution was normal; therefore, paired t-test was used. The results of the t-test are listed in Table 2.

Table 2. Significance of the pixel grey value before and 4 weeks after iRoot® BP Plus application

| Carious tissue removal                  | N  | Mean (SD)      | p value* |
|----------------------------------------|----|----------------|----------|
| Infected dentin partially removed      |    | 0.000          |          |
| Before treatment                       | 5  | 123.40 (8.691) |          |
| After 4 weeks                          | 5  | 151.40 (9.737) |          |
| Infected dentin entirely removed       |    | 0.002          |          |
| Before treatment                       | 5  | 168.80 (17.541)|          |
| After 4 weeks                          | 5  | 195.40 (25.265)|          |

* There is significant difference P<0.05

In Table 2, there was a significant difference before and 4 weeks after iRoot® BP Plus application in the infected dentin partially removed group (p ≤ 0.05) and in the infected dentin entirely removed group.

In Table 3, by comparing the increase in the pixel grey value for both treatment groups, the result showed no statistically significant difference between the two treatment groups (p ≤ 0.05).

Table 3. Significance of the comparison of pixel grey value increase between treatment groups 4 weeks after iRoot® BP Plus application

| Carious tissue removal                  | N  | Mean (SD)      | p value* |
|----------------------------------------|----|----------------|----------|
| Increased grey value in group with infected dentin partially removed after 4 weeks | 5  | 151.40 (9.737) | 0.14     |
| Increased grey value in group with infected dentin entirely removed after 4 weeks     | 5  | 195.40 (25.265)| 0.05     |

P<0.05

4. Discussion
In deep carious lesions, the remaining dentin thickness is approximately one-fourth of the normal dentin thickness. In such cases, the complete removal of the carious tissue may cause a risk of pulp
The present study, single-visit excavation of caries was performed, leaving only affected dentin, and leaving infected and affected dentin. Both methods were selected to protect the dental pulp from exposure. Based on the study by Schwendike et al. single-visit selective excavation has a smaller risk of opening the pulp than two-visit selective excavation [3].

In the present study, dentin layer determination was done using caries detector dyes. This material was used to distinguish between affected and infected dentin; therefore, the limit of selective caries tissue removal could be determined and the risk of bias could be reduced in the present study.

In the treatment of vital pulp, bioactive materials play an important role in eliminating bacteria, providing adequate closure, and triggering mineralization [22]. To maintain the vitality of the pulp and prevent pathological changes, caries tissue should be covered with biocompatible materials to protect the pulp from injury and to trigger healing and repair. One of the silica-based cements used in the present study is iRoot® BP Plus. Remineralization capability using iRoot® BP Plus in vitro has been associated with the ability to release calcium ions. The size of the bioerodible particles is <2 microns, and the small size increases the surface area of the exposed material, which effectively increases the release of calcium ions and at the same time, enhances remineralization to intrafibrillar remineralization. In addition, Zhang et al. found that iRoot® BP Plus significantly improves healing of pulp tissue in mice [16]. iRoot® BP Plus shows better biocompatibility and osteoconductivity ability than MTA.

The results of the present study are also in line with the results from the 2011 research of Gandolfi et al. who examined in vitro remineralization using bioactive materials. Based on this study, the removal of calcium ions from the material triggers the occurrence of remineralization. In fact, using 2–20 μm of material, the newly formed apatite crystals bonded closely to the dentin structure rather than simply being deposited on the surface of the lesion [23].

If there are still collagen and non-collagen proteins, such as DMP1 and DMP2, in dentin, there is a possibility for intrafibrillar remineralization as they are regulators for nucleation and mineral growth processes. The non-collagen protein is known to regulate the biomineralization of dentin in vivo [24].

Therapy to improve remineralization can be evaluated by determining changes in the quality and quantity of minerals in the teeth. Potential diagnostic tools, such as OCT and digital radiographs, provide an overview of dental remineralization. OCT is a high-resolution, non-invasive method and produces three-dimensional images. However, this device has limited scanning and is not yet available in Indonesia [17, 18].

Prati et al. examined the biological perspectives of calcium silicate bioactive cement that was immersed in a simulated body fluid and analyzed using SEM/EDX. It was reported that after 28 days, there was an increase in apatite, and the intensity increased over time [21]. Therefore, in the present study, increasing grey value by digital radiograph examination was expected so that the rate of remineralization after 4 weeks could be calculated using the Digora™ Optime tool.

Table 2 shows that the increase in the pixel grey value in both treatment groups is statistically significant. Thus, the first and second hypotheses are accepted, which state that there is remineralization of affected dentin in deep carious lesions with partially removing and entirely removing infected dentin closest to the pulp 4 weeks after iRoot® BP Plus application.

In some studies, it has been reported that it is impossible to eliminate all microorganisms despite the complete removal of the carious tissue, because some bacteria remain, although all soft dentin has been removed. However, the pulp continues to defend against bacterial acids and toxins. Therefore,
restoration with good density and use of bioactive materials increases the dentin’s potential for remineralization and maintains the survival of the pulp.

Tian et al. reported that the release of silicon and calcium ions from iRoot® BP Plus is higher at pH 5.4 than at pH 7.4. The higher the ion release, the higher is the solubility, thus affecting the sealing ability and the micro hardness of the material. At a low pH, compared with Pro Root MTA®, iRoot® BP Plus has a homogeneous, superficial microstructure with thin, non-porous crystal sheets. This structure has been described as a nanoparticle component in iRoot® BP Plus. The acid environment interferes with the formation of apatite but still encourages the viability of MC3T3-E1 pre-osteoblast cells [15]. Based on this research, it can be concluded that remineralization still occurs in affected dentin with acidic atmosphere, although some of the infected dentin remains. In addition, the osteoconduction capacity of iRoot® BP Plus material is higher than that of MTA, and it has nano-sized particles; therefore, remineralization of affected dentin is higher.

From the statistical testing using unpaired t-test, there was no significant difference between the two groups after iRoot® BP Plus application, with p = 0.14 (p > 0.05). In other words, there was no statistically significant difference in dentin remineralization rates between caries tissue removal techniques by leaving some of the infected dentin or by removing all infected dentin after iRoot® BP Plus application. Thus, the third hypothesis is accepted wherein there is no difference in the remineralized rate of affected dentin in deep carious lesions by leaving a portion of the infected dentin at the nearest portion of the pulp or by removing all infected dentin in the one-step partial excavation technique after iRoot® BP Plus application for 4 weeks.

The results of the present study are in line with the results of a report by Ricketts et al., who evaluated the activity of lesions that had been restored by leaving infected dentin, showing the lesion to be inhibited in both clinical and radiographic development [5]. Affected dentin can be left at the base of the cavity and may undergo remineralization, thus preventing the opening of the pulp. Therefore, removal of the entire infected dentin in caries is not necessary to achieve successful caries treatment. The presence of the infected dentin layer was not a concern, particularly the presence of an advanced caries process. The increased grey value of radiographic examination showed not only that the caries process stopped but also that dentin remineralization occurred, although the change was very small. This was in accordance with several studies of caries disease by Kidd (2004), Bjorndal and Kidd (2005), Kidd and Fejerskov (2004), and Marsh (2006) that stated that ecological changes in the carious lesion area can stop the caries process without eliminating cariogenic bacteria [9-10,14,25].

Based on the acceptance of the third hypothesis, it can be concluded that the partial caries excavation technique with infected dentin partially removed and the application of bioactive materials can be a caries removal technique chosen by the dentist in accordance with the minimal intervention philosophy in the case of deep carious lesions to trigger the process of remineralization on the dentin while maintaining the vitality of the pulp.

5. Conclusion
Remineralization of affected dentin was observed in the group with partially removed infected dentin nearest to the pulp and the group with all infected dentin removed 4 weeks after iRoot® BP Plus application. There was no difference in the remineralization rate of affected dentin in deep carious lesions between the two groups.

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