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The effect of MTA application on the affected dentine remineralization after partial caries excavation (*in vivo*)

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Abstract. On deep carious lesions, only thin dentine remains, causing a high risk of pulp exposure during the removal of all infected dentine. A minimally invasive technique is required, such as a partial caries excavation method in the infected dentine tissue and the use of bioactive material that can promote (Mineral Trioxide Aggregate) MTA remineralization. To compare the remineralization of deep carious lesion-affected dentine with the removal of some and all the infected dentine after the application of MTA. Subjects were divided into two groups: group I had only some parts of the infected dentine removed before MTA application, while group II had all the infected dentine removed before MTA application. Each group was measured on the pixel grey value before the treatment and again four weeks after the MTA application, and then the results were compared. Furthermore, the enhancement of both groups' grey values were compared. Remineralization occurred in both groups after the MTA application. There was no significant difference in the remineralization level of the affected dentine in both groups I and II four weeks after the MTA application. Remineralization occurred in the affected dentine in both groups, either by removing only some parts or all the infected dentine in the deep carious lesion.

1. Introduction

In 1893, GV Black proposed the principle of carious treatment based on the concept of "extension for prevention," which is the disposal of carious tissue with the aim of retention for restoration and prevention. Such disposal techniques are called complete caries [1]. Around 2002, there was a change in the concept of caries treatment, favoring the removal of all infected dentine, and the removed dentine was abandoned. This technique is still common today. However, this procedure is too expensive and not recommended in deep carious lesions [2]. In deep carious lesions, the remaining thickness of residual dentine is extremely thin, i.e., 25% of normal dentine thickness [3]. Therefore, removal of all infected dentine has a high risk of pulp exposure [3]. Studies have shown that it is not possible to remove all bacteria in a carious lesion. Thus, a new understanding of caries removal principle developed: a caries is not an infectious disease that requires total removal. Removal of all bacteria in a caries lesion is not necessary; simply by altering the ecological and metabolic balance of biofilm, remineralization may occur and the carious lesion is stopped [4].

The availability of bioactive materials, such as MTA, is thought to stimulate remineralization, which can lead to healing [5]. The material may emit an ion that can induce precipitation or apatite crystal formation above partially demineralized collagen matrices [6]. MTA can interact with dentine during intrafibrillar deposition [7]. MTA has high alkaline pH, antibacterial, biocompatibility, radiopacity, and good closure properties [5,8]. In 2016, based on the new understanding and material progress, the International and American Associations for Dental Research (IADR) in the International Caries Consensus Collaboration (ICCC) introduced the technique of selective removal of soft dentine, which is defined as a caries removal technique, by leaving the soft dentine, i.e., some parts of the infected dentine and all affected dentine [4]. This is done to maintain pulp vitality.

Some of these caries removal methods include two-step visits, or "stepwise," and one-step visits, or one-step step caries removal [4]. In stepwise excavation, selective removal is performed on the first visit, and then the opening is done again on the second visit. In one-step excavation, the selective removal, a liner, and the filling applications were performed in one visit [9]. Maltz *et al.* [9] suggested that survival rates of one-step excavation after one and three years were 98% and 91%, and success rates of treatment were 97% and 90%, respectively. There are several ways to calculate remineralization of dentine. One method is using digital radiography. Digital radiography is the most widely used method for detecting carious lesions and for evaluating measurable treatments through gray tones or gray values. This is based on the theory that the human eye can only see 32 gray tones, while the computer can identify 256 gray tones. Some computer programs are used to manipulate digital images, including Adobe Photoshop®, Digora®, Image Tool®, and VixWin® [10]. Based on research by Nobrega *et al*. [11], the Digora radiography system can be used to measure density with sufficient degrees of sensitivity. Based on research by Salzedas [12], CCD gives the same image display as the Digora PSP system, while Akcay [13] mentioned that the PSP image is better than CCD.

Remineralization of dentine can occur through a biomaterial application that begins with the formation of mineral foci. This study was carried out for four weeks because the formation of mineral foci began two days after application, and at days 14 and 28, the number and form of minerals continued to increase. In addition, based on observations using SEM and EDX, spherulite minerals formed seven days after application of bioactive materials. On the 28th day, silica was undetectable and there was an increase in calcium and phosphate [14]. Thus, it is assumed that with an increase in apatite, the density increases and can be seen with digital radiography DigoraTM Optime. Therefore, this paper's purpose is to examine the effect of MTA application on affected dentine remineralization after partial caries excavation in-vivo by measuring the four-week remineralization using pixel gray value with DigoraTM Optime digital radiography**.**

2. Materials and Methods

The method of collecting subjects is done consecutively, which is collecting a number of subjects within a certain time limit. In this study, the sample collection limit was for one month. Within one month, the number of samples for each treatment group was five subjects. In this study, there were two treatment groups; thus, it is determined that the number of samples was as many as 10 subjects.

Research subjects were patients who came to Teaching hospital Faculty of Dentistry Universitas Indonesia with premolar 1/premolar/molar 1/molar 2 cavities without any spontaneous pain. Tooth vitality examination with positive thermal (ethylchloride) test, and negative percussion. Cavity was site 1/site 2 or Black class 1/class 2. On radiographic images, subjects with deep carious lesions and showed a remaining normal dentine thickness of 0.25 to 1 mm from pulp. Subjects were randomly selected for inclusion in group I or group II. Patients were informed about the study and signed informed consent forms.

In groups I and II, the tooth caries was isolated with a rubber dam. Detector dye was applied to the tooth caries (SableTM Seek®, Ultradent, USA). Then the selection was performed. In group I, the dark green part nearest the pulp was confirmed through radiography, and the excavation of some superficial dentine caries (some infected dentine) with a sterile EXC 63-64 (Osung, South Korea) excavator was performed. On the cavity wall and floor, which were still far from the pulp, excavation was done of all the infected dentine, and the affected dentine, which was marked by the light green color of caries detector dye, was left. In group II, on the dark green part, excavation was done of the entire infected dentine tissue with a sterile EXC 63-64 (Osung, South Korea) excavator until the caries detector dye was light green. The teeth were then cleaned and dried. An application of 2 mm MTA on

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the cavity floor, using a ball-pointed PUNC15 periodontal probe (Osung, South Korea), was done. Next, an application of GC Fuji IX (GC Corporation, Japan) was done until it covered the entire cavity. Patients were instructed to return within four weeks.

Four weeks after treatment, radiographic photographs were taken with the same angle as the initial photographs. The density measurement was then done with Dormore Soredex DigoratmTM Optime (Soredex Corp., Tuusula, Finland) and calculated using the computer software system. The measured densities before and after treatment were compared. The data obtained were analyzed statistically with SPSS ver. 20. A statistical test using dependent t-test was performed in each group before and after treatment. Meanwhile, to compare the results of both groups after treatment, an independent t-test was done if the data were normal.

3. Results and Discussion

3.1 Results

Based on the observation of all subjects, remineralization occurred in each group, whether there was only partial or complete removal of the infected dentine. This is shown in Table 1.

Table 1. Table of means for the pixel gray dentine on partial and complete removal of infected dentine before and four weeks after MTA application.

Removal of carious tissue	*Pixel gray value*			p-value*
	N	Before treatment (Means±SD)	Four weeks after treatment (Means±SD)	
Infected removed partially	5	130.20±6.221	137.20±8.408	0.035
Infected removed completely	5	141.40±5.128	153.60±7.232	0.020

*) Paired t-test

Table 1 shows the gray pixel values in both groups, either with partial dentine removal or before and one week after MTA application. Based on a data distribution test with Shapiro-Wilk, the data distribution obtained was normal in all groups; thus, a paired t-test was done. The result shows significant differences between before and four weeks after MTA application in both groups in teeth with partially or unconfirmed infected dentine ($p \leq 0.05$). Table 2 shows the comparison of gray value increase between treatment groups, which was an unknown result between two treatment groups ($p \leq 0.05$).

Table 2. Significance of pixel gray value increase between treatment groups after four weeks of MTA application.

	N	Means (SD)	p-value*
Increase of pixel gray value in group with partial removal of infected dentine	5	137.20 (8.408)	0.081
Increase of pixel gray value in group with complete removal of infected dentine	5	153.60 (7.232)	

3.2 Discussion

In this research, pixel gray value measurement is done using the Digora™ Optime system. Based on research by Nobrega *et al*. [11], the Digora radiography system can be used to measure density with sufficient degrees of sensitivity. Based on research by Salzedas [12], CCD gives the same image display as the Digora PSP system, while Akcay [13] mentioned the PSP image is better than CCD [11]. In a previous study, the experimental procedure was standardized with the use of a photo bite device in the form of a bite registration of a polyvinyl siloxane material used as a bite registration of the subject during a radiographic photograph [15]. Thus, in this research, bite registration is also used as a record so that radiographic photographs before and four weeks after treatment can be taken with the same position and bite.

In this study, the caries removal method was done in one visit (one-step excavation). This is done to reduce the risk of opening the pulp [9]. Maltz *et al*. [9] stated that the survival rates of one-step excavation after one and three years were 98% and 91%, and the success rates of treatment were 97% and 90%, respectively. Compared with stepwise excavation, survival rates of partial removal of carious tissue were similar; however, this technique had a lower risk of failure than the stepwise technique within three years [9]. In this study, a selective excavation technique was performed, in which dye caries detector (SableTM and Seek®, Ultradent, USA) was used to distinguish infected and affected dentine. Thus, selective excavation boundaries may become more pronounced to reduce the risk of bias in this study [16].

Tricalcium in MTA is a major component that is biocompatible and bioactive [7]. MTA can remove calcium and hydroxyl ions that form hydroxyapatite crystals on dentine surfaces. MTA can also interact with dentine during intrafibrillar deposition [7]. MTA has high alkaline pH, antibacterial, biocompatibility, radiopacity, and good closure properties [5,8]. MTA can release calcium ions; Sarkar *et al*. [17] stated that the dominant calcium ions released from the MTA will react with phosphates in synthetic tissue fluids and form hydroxyapatite. The coated layer between dentine and MTA will also produce a reaction similar to that of the experiments performed [17]. Asrianti [18] conducted a study that examined the removal of calcium ions from ProRoot MTA® SIK. The study's results showed 45% calcium ion release within 15 minutes after the initial setting and 53% within 30 minutes after the initial setting [18]. Rodrigues *et al*. [19] conducted a study of ion release from MTA, calcium hydroxide, and Biodentine® that found MTA releases more calcium ions compared to Dycal® and Biodentine® at a pH of 5.5 [20]. Calcium silicate cements, such as MTA, not only have the ability to remove calcium and hydroxyl ions after contact with cells and tissue fluids but can also form apatite hydroxy crystals on the surface. The formation of apatite can reduce leakage not only in the gap between the filling material and the cavity but also interact with dentine during intrafibrillar apatite deposition. The characteristics of this layer formation, in terms of composition and structure, are similar to that of hydroxyapatite when placed on dentine. A good closure of MTA is initially received physically and then becomes chemically bound thereafter [21].

MTA has anti-bacterial properties, and MTA has a pH of 10.2 after mixing that rises to 12.5 after setting [22]. MTA releases calcium ions during the setting reaction, and the important thing is to provide an alkaline pH. A prolonged alkaline pH of 12.5 provides the potential antibacterial and antifungal properties [7]. Table 1 shows remineralization occurred in both groups, either with the removal of some infected dentine or with the removal of all the infected dentine. In this study, the remineralization rate was determined based on pixel gray value four weeks after the removal of the infected dentine. This is based on research by Prati *et al*. [14], which stated that there is an increase in appetite for 28 days on bioactive cement calcium silicate saturated in a simulation of body fluids, in which longer durations produced increased intensity [14]. There were statistically significant differences in the increase of pixel gray values for the two treatment groups. Remineralization of affected dentine in deep caries lesions occurred by removing part and all the infected dentine four weeks after MTA application.

Remineralization occurs if at least 10% of the minerals remain [23]. In addition, the presence of collagen and non-collagen proteins (NCPs) in the dentine matrix causes the remineralization process by MTAs to occur. NCPs play an important role in regulating the remineralization process [24]. NCPs with high affinity to calcium and collagen ions play a role in nucleation and crystal growth. Examples of such proteins include dentine matrix protein (DMP1) and dentine phosphophoryn (DPP, DMP2).

DMP1 can form prenucleation clusters and amorphous calcium phosphate (ACP) nanoparticles that are placed inside collagen fibers, particularly within the gap zone. With these capabilities, DMP1 is an important NCP for biomimetic remineralization [25].

MTA can remove calcium and hydroxyl ions that form hydroxyapatite crystals on the dentine surface. MTA can also interact with dentine during intrafibrillar deposition [7]. MTA has high alkaline pH, antibacterial, biocompatibility, radiopacity, and good closure properties [5,8]. With a selective caries removal method accompanied by the application of MTA material to deep carious lesions, the process of remineralization may occur. From the statistical test using an unpaired t-test, there was no significant difference between the group that removed some of the infected dentine and the group that removed all the infected dentine four weeks after MTA application, with a significance value of $p = 0.081$ ($p > 0.05$) remineralization rate of affected dentine in deep caries lesions after partial removal of infected dentine with MTA application is equal to that after complete removal of infected dentine with an application of MTA for four weeks.

This study's results are in line with a report by Ricketts *et al*. [3] that evaluated the activity and development of lesions that had been restored by leaving the infected dentine, and the results showed clinical and radiographic progress. Therefore, removal of all infected dentine in deep caries is not necessary to achieve successful caries treatment. Kuhn *et al*. [26] mentioned that arrested caries in dentine occurred after liner material was placed on the affected dentine. The technique of one-step excavation by leaving part of the infected dentine with an application of bioactive MTA material in areas close to pulp may be a choice for caries removal techniques in deep carious lesions. This technique is in accordance with the philosophy of minimal intervention in cases of deep carious lesions to trigger the process of remineralization in the affected dentine. In addition, this technique can prevent pulp exposure so that pulp vitality can be maintained.

4. Conclusion

From this study, it was concluded that after four weeks of MTA application there was remineralization of affected dentine in both groups, whether there was partial or complete removal of the infected dentin. After four weeks of MTA application, there was no difference in remineralization rates between the groups that had partial or complete removal of the infected dentine.

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