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Differences between micro-hardness affected dentin after mechanical or chemo-mechanical infected dentin disposal (laboratory experiment)

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Abstract. The concept of conserving healthy tooth structures during cavity preparation has gained popularity with chemo-mechanical caries removal. This study compared three methods of caries removal using: a chemo-mechanical caries removal papain gel; Papacarie® (these contain natural ingredients, mainly papain enzyme); and mechanical preparation with a bur rotary instrument. The purpose of this study was to compare affected dentin micro-hardness after removal of infected dentin with mechanical and chemo-mechanical techniques. Twenty-seven permanent molar teeth were randomly divided into three groups receiving removal of infected dentin. These were: Group 1: chemo-mechanical technique using papain gel; Group 2: chemo-mechanical technique using Papacarie®; Group 3: mechanical technique using a bur rotary instrument. Each group was tested using Knoop Micro-hardness tester, and the data were submitted to one way ANOVA and Post-hoc Tukey test. There is a significant difference between Groups 1 and 3, and Groups 2 and 3, p = 0.000. However, there is no significant difference between Groups 1 and 2, p = 1.000. Affected dentin micro-hardness after removal of infected dentin with a bur rotary tool is higher than after use of the papain gel or Papacarie®. Affected dentin micro-hardness after removal of infected dentin with Papacarie® and papain gel give almost the same result.

1. Introduction
The concept of maintaining a healthy dental tissue structure has now grown to refer to the principle of minimal intervention. Minimum intervention philosophy includes measures of caries risk assessment, early caries detection, early demineralization of the lesions, minimal preparation of the cavity, and restoration of damaged areas [1,2]. In the past, removal of carious tissue followed the principle of cavity preparation based on the concept of "extension for prevention", i.e. removal of carious tissue while considering retention for restoration and prevention of secondary caries. For this reason, healthy dentin tissue retrieval often became redundant. Through time, dental care networks developed the principle of patient-friendly minimum preparation, in accordance with minimal interventionist philosophy. Several methods have been developed in accordance with the principle of minimum preparation such as laser preparation, air abrasion, and chemo-mechanism. These methods can serve to calm patients who experience excessive fear, reduce discomfort, and minimize anesthetic requirements [3].
The current minimal intervention philosophy developed at dental care networks uses the principle of "prevention of extension", a practice that emphasizes the prevention of excessive dentin dental disposal by simply removing the infected dentin layer and leaving a dentin-affected layer where the collagen fibers are intact, allowing them to be remineralized. Remineralization of the affected dentin layer may occur after the cavity is coated with a glass ionomer cement material. Chemo-mechanical preparation with Carisolv® was introduced in 1998; this was an early development of chemo-mechanical products. Carisolv® contains sodium hypochlorite, and amino acids (glutamate, leucine, and lysine). However, its application requires a long processing time, making patients less comfortable; it is also difficult to obtain as well as expensive. Another chemo mechanical ingredient, Papacarie®, was introduced in Brazil in 2003; it contains natural papain ingredients along with chlorine and toluidin blue. The use of the chemo-mechanical technique with papain gel as the main ingredient of the Papacarie® product is currently highly developed.

The main content of papain (found in both Papacarie® and papain gel) is papaya enzyme from the species Carica papaya, widely found in various tropical countries such as Indonesia. Papain is bactericidal and bacteriostatic, can act as an anti-inflammatory agent, does not damage healthy tissue, has a faster processing time, and is more affordable in cost [4]. Previous study showed that papain has an effect only on infected tissue, reacting by cutting collagen molecules that have been damaged by the caries process [5]. In Indonesia, Papacarie® is yet not available on the market, while papaya from the Carica papaya species grows widely and is easy to obtain. In this study we compared the removal of caries tissue with mechanical techniques using a bur rotary tool versus chemo-mechanism using Papacarie® or papain gel. The study used small specimens, so the measurements are made using a micro-hardness test with Knoop indentation, because it can be used on a thin dental specimen with a narrow surface.

2. Materials and Methods
Twenty-seven extracted molar teeth, with caries reaching dentin and completely closed apical parts, were cleaned under running water and then stored in a plastic container with saline solution until the study was conducted. At that point, the sample was randomly divided into three groups. The first group of 9 teeth had infected dentin removed using papain gel (Group 1). The second group of 9 teeth had infected dentin removed using Papacarie (Group 2). The third group of 9 teeth had infected dentin removed using a bur instrument (Group 3). Dental specimens for all groups were dried. For Groups 1 and 2, Papacarie® or papain gel was applied into the cavity for 30-40 seconds. Then, the removal of infected dentin was done on the softened part, using the excavator with a swinging motion like a pendulum; soft tissue was excavated with screws. The specimens in Group 3 were treated with a swivel instrument using a spherical steel bur with a low speed hand piece for removal of the infected dentin layer, until clinical signs of the affected dentin layer, such as leathery texture, were obtained. Each specimen was rinsed with water spray, and then the cavity was dried with cotton pellets. Next the dental specimens were planted perpendicularly inside the PVC cable duct by using a hardener with added decorative resin. After this, the dental specimens were split into two parts, namely the mesial and distal. Then the crown of the tooth was separated from the root using a carborundum disc with low speed. Each dental specimen was labeled according to its grouping.

Subsequently, the specimen pieces were once again planted in a decorative resin mixed with a hardener, with the surface to be measured for hardness planted face upward in a paralon water pipe mold. After the resin hardened, the resin and teeth were removed from the mold. The surface of the tooth specimen was polished by a grinding or polishing machine until a flat surface on the specimen was obtained. The specimens were immersed in a saline water solution until a micro-hardness measurement was performed. Measurement of micro-hardness on dentin was performed at 5 dentin-affected depth points with distances of 0 μm, 50 μm, 100 μm, 150 μm, and 200 μm. At each depth, indentation using a hardness tester was applied three times. One way ANOVA test was used to observe the difference between the three groups, followed by a Post-Hoc test to observe the differences between each set of two groups.
3. Results and Discussion

3.1 Results
This study analyzed the hardness of the affected micro-dentin at five depth points below the cavity after removal of infected dentin using a mechanical technique with a bur instrument, or chemomechanical techniques using Papacarie® or papain gel. Measurements were performed using a micro-hardness tester tool with Knoop indenter, obtained by means of dentin induced hemorrhagic violence from the 15 groups listed in Table 1 below.

Table 1. Distribution of mean values of dentin-affected (KHN) hardness at five depth points below the cavity, after removal of infected dentin using papain Papacarie®, or bur

| Group      | Depth Points | Mean (KHN) ± SD | CI 95% |
|------------|--------------|----------------|--------|
| Papain     | 0 µm         | 11.19 ± 4.19   | 7.95 - 14.39 |
|            | 50 µm        | 12.88 ± 3.89   | 9.89 - 15.87 |
|            | 100 µm       | 14.58 ± 4.08   | 11.44 - 17.71 |
|            | 150 µm       | 16.54 ± 3.79   | 13.62 - 19.46 |
|            | 200 µm       | 18.56 ± 3.78   | 15.64 - 21.46 |
| Papacarie® | 0 µm         | 11.40 ± 3.33   | 8.83 - 13.96 |
|            | 50 µm        | 13.34 ± 3.20   | 10.87 - 15.80 |
|            | 100 µm       | 14.87 ± 3.59   | 12.10 - 17.63 |
|            | 150 µm       | 16.96 ± 3.44   | 14.30 - 19.60 |
|            | 200 µm       | 18.33 ± 3.46   | 15.66 - 20.99 |
| Bur        | 0 µm         | 32.10 ± 11.20  | 23.48 - 40.71 |
|            | 50 µm        | 38.59 ± 10.37  | 30.61 - 46.56 |
|            | 100 µm       | 41.50 ± 10.42  | 33.48 - 49.51 |
|            | 150 µm       | 42.72 ± 9.74   | 35.22 - 50.21 |
|            | 200 µm       | 45.27 ± 10.87  | 36.90 - 53.62 |

Table 1 shows the data on the mean value of dentin-affected micro-hardness at five points of depth for the three groups, namely Group 1 (papain), Group 2 (Papacarie®), and Group 3 (bur). For all depth points, the mean values of dentin-affected micro-hardness of Group 1 did not differ greatly from Group 2. However, the mean values of dentin-affected micro-hardness for Groups 1 and 2 were lower than those for Group 3. In this study, the normality of data was tested using Kolmogorov-smirnov test, obtaining p value > 0.05, which means the distribution of normal data. After the distribution of data was determined to be normal, a parametric test was performed, that is a hypothesis test using ANOVA and Post-Hoc analysis, to show each meaningful group, and then testing was continued with the Tukey test. The results of the ANOVA test for dentin-affected violence at all points of depth for all three groups were p = 0.000. From the data obtained p-value < 0.05, it can be concluded that there are significant differences for the hardness of micro-dentin affected micro-hardness after removal of infected dentin in all three groups (papain, Papacarie®, and bur) for all depth points.

Table 2 shows that the data for dentin-affected micro-hardness after removal of infected dentin for Group 1 (using papain) revealed no statistically significant difference between the depth points of 0 µm compared with the other four depth points. This shows that the value of dentin-affected micro-hardness for Group 1 (papain) at depths of 0 µm, 50 µm, 100 µm, 150 µm, and 200 µm was almost the same. Table 2 shows that the data for dentin-affected micro-hardness after removal of infected dentin for Group 2 (using Papacarie®) revealed no statistically significant difference between the depth point of 0 µm compared with four other depth points. This shows that the value of dentin-affected micro-hardness for the Papacarie® group at depths of 0 µm, 50 µm, 100 µm, 150 µm, and 200 µm was...
Table 2. Significant value (p) of dentin-affected micro-hardness in papain, Papacarie®, and bur groups at five points of depth

|                  | Depth Points μm |       |       |       |
|------------------|-----------------|-------|-------|-------|
|                  | 50              | 100   | 150   | 200   |
| Papain 0 μm      | 0.996           | 0.656 | 0.087 | 0.050 |
| Papacarie 0 μm   | 0.995           | 0.748 | 0.124 | 0.021 |
| Bur 0 μm         | 0.966           | 0.702 | 0.501 | 0.235 |

Anova test with significance p < 0.05

similar. Table 2 shows that the data for dentin-affected micro-hardness after removal of infected dentin for Group 3 (using bur) revealed no statistically significant difference between the depth point of 0 μm compared with the other four depth points. This shows that the value of dentin-affected micro-hardness for bur groups at depths of 0 μm, 50 μm, 100 μm, 150 μm, and 200 μm is similar. The value of dentin-affected micro-hardness after removal of infected dentin in Group 1 (the papain group) compared with Group 2 (the Papacarie® group). There was no statistically significant difference in all depth points. This shows that the value of dentin-affected micro-hardness at all points of depth between the papain and Papacarie® groups is almost the same. The value of dentin-affected micro-hardness after removal of infected dentin in Group 1 (the papain group) compared with Group 3 (the bur group). There was a statistically significant difference in all depth points. This shows that the value of micro-hardness of dentin-affected of the papain group at all depth points is lower than that of the bur group. The value of dentin-affected micro-hardness after removal of infected dentin in Group 2 (the Papacarie® group) compared with Group 3 (the bur group). There was a statistically significant difference in all depth points. This shows that the value of micro-hardness of dentin-affected of the Papacarie® group at all depth points is lower than that of the bur group.

3.2 Discussion

The objective of this study was to analyze the differences in dentin-affected layer microstructure after removal of infected dentin using a mechanical technique (i.e. bur instrument), or chemo-mechanism with Papacarie® or papain gel. Measurements were performed at five depth points; those were 0 μm, 50 μm, 100 μm, 150 μm, and 200 μm. The teeth used were fixed molar teeth for which extraction was indicated due to periodontal abnormalities. The selection of dental specimens includes the maxillary and lower left molar teeth, since it is the first permanent eruption tooth and often has the most caries. The caries depth criteria reached dentin in the pit, fissure, and proximal regions, and had no abnormalities in the enamel and dentin structures, and the teeth were free of patches. Dental specimens were stored in a saline solution until the study was conducted, which was expected to represent the same moisture conditions as the physiological conditions in the oral cavity, thus not affecting dentin hardness. In carious lesions, infected dentin was removed with mechanical techniques using bur instrument or chemo mechanical techniques with papain gel or Papacarie®. (The reason for selection of mechanical techniques with the bur is that this technique has historically been done for the removal of caries lesions.) The chemo-mechanical technique was performed to observe the dentin-affected violence after the dentin infected removal. The Chemo-mechanical removal method was used because it was in accordance with minimally invasive principle.

Preparation for the chemo-mechanical technique was done through minimally invasive removal of the carious tissue using a chemical agent. One of the chemo-mechanical preparation products was Papacarie®, which was introduced in 2003 in Brazil. This product contains mainly papain, chlorine, and toluidine blue. The main natural ingredient of papain is made from papaya enzyme from the species Carica papaya. Carica papaya is widely grown and easily obtained in Indonesia, so papain enzyme production could also be done. Papain interacts with collagen exposed by breaking the mineral dentin through the bacteria, making the infected dentin softer [6]. In this study, a micro-
hardness test was performed because it is generally easy to do on small specimen sizes. The Knoop indentation method was chosen because it does not damage the specimen and can be used on thin specimens and narrow surfaces. The surface of the tested specimen was smooth and clean, and perpendicular to the indenter [7]. The load used in the micro-hardness measurement was 25 gf for 10 seconds. The load was considered sufficient to permanently deform the tooth structure to be measured. Indentation times between 10, 20, and 30 seconds do not make a significant difference to the enamel hardness value of dentin and enamel when using the same load, so 10 seconds was considered sufficient to produce a permanent indentation on the tooth surface [8].

The mean values of dentin-affected microstructure varied for each group and tended to be lower than normal dentin micro-hardness values. This is in accordance with a 2007 study by Bresciani et al., which stated that normal dentin hardness values ranged 53-80 KHN, with higher dentin micro-hardness values in deep dentin layers [8]. Variations in dentin hardness are influenced by several factors, including dentin calcification rate, and difference of dentin density at various sites. On the other hand, the mean value of dentin-treated micro-hardness of dentin after removal of infected dentin for all three groups at all depths was consistent with the value of dentin-affected micro-hardness presented in previous study, ±13.64+48, 35 KHN [8,9]. Measurements were made by indenting at five depth points starting from 0 μm representing dentin closest to the cavity, then 50 μm, 100 μm, 150 μm, and 200 μm. At each depth, three longitudinal lesions were made. For each depth three dentin micro-hardness values (KHN) were averaged to obtain the dentin micro-hardness value representing the point of hardness. This measurement procedure is in accordance with research by Kato and Fusayama, which states that the measurement of dentin micro-hardness can be performed on dentin from the bottom of the cavity to a depth of 200 μm [10].

Based on the results of this study, it was found that the mean value of dentin-affected micro-hardness in the bur group was higher for all depth points than the mean values for the papain and Papacarie® groups. This is consistent with a 2007 study by Fernandea et al., suggesting that removal of carious tissue using chemo-mechanical techniques would leave the most dentin-affected layers in comparison with mechanical techniques [3]. The above results support the current opinion that the removal of carious tissue should be minimized, thereby reducing the risk of wasting too much healthy dentin tissue which in time can lead to fractures. Minimally invasive philosophies include measures of caries risk assessment, early caries detection, early demineralization of the lesions detected, minimal preparation of the cavity, and restoration of damaged areas [1,2]. Cavity preparation of carious lesions is no longer done using the principle "extension for prevention" developed by G. V. Black. This has been changed to "prevention of extension" as developed by G. J. Mount. The principle of removing previous caries tissue, as the preparation must produce a hard cavity surface, can in fact lead to the excessive loss of healthy dentin tissue which can cause fractures and trigger the opening of the pulp chamber [11].

Currently, there are adhesive restoration materials such as glass ionomer cement which can trigger remineralization. The closure of the cavity after removal of the carious tissue in the dentin-affected layer with an adhesive restoration material such as glass ionomer cement can reinforce the tooth [11]. Thus, the current minimal preparation principle has been widely applied. Based on research on the papain gel and Papacarie® groups, the hardness of the outermost dentin layer at a depth of 0 μm has the lowest hardness value compared to the other four depth points. This is consistent with a 2008 study by Qasim et al., which states that dentin hardness in the outermost layer is lighter than in the deeper layers, as papain gel and Papacarie® selectively damage and dissolve only the infected dentin layer, leaving the affected dentin [12]. At depths of 50 μm to 200 μm, the dentin-affected micro-hardness values in the papain and Papacarie® groups increased from a depth of 0 μm. This is in accordance with the research of Sakoolnamarka et al. (2005), which states that the value of micro-hardness is influenced by the calcium and phosphate content that will increase at a depth of 100 μm to 1000 μm compared with a depth of 0 μm [9].

The results of the bur group studies at 0 μm depth showed the lowest dentin-affected micro-hardness compared to the other four depth points. In this study, although there were differences in
mean values of micro-hardness at all points of depth from 0 μm to 200 μm for each group, there was no statistically significant difference. The value of dentin-affected micro-hardness after discharge of infected dentin in the papain gel group compared to the Papacarie® group, showed no statistically significant difference in all depth points. Dentin-affected micro-hardness after removal of infected dentin with Papacarie® was similar to that found with papain gel application. It was concluded that the papain and Papacarie® groups had the same effect in leaving the soft tissue on the dentin.

The value of the dentin-affected micro-hardness after discharge of infected dentin in the papain group compared with the bur group was statistically significant in all depth points. This result is consistent with the second research hypothesis which states that the value of dentin-affected micro-hardness after discharge of infected dentin with a bur is higher than after papain gel application. It was concluded that the chemo-mechanical technique using papain gel still leaves more affected dentin layer than does the mechanical technique using bur. Similarly, the value of dentin-affected micro-hardness after discharge of infected dentin in the papain and Papacarie® groups compared with the bur group revealed a statistically significant difference in all depth points. This result is consistent with a third research hypothesis which states that the value of dentin-affected micro-hardness after removal of infected dentin with a bur is higher than after the Papacarie® application. It was concluded that the chemo-mechanical technique using Papacarie® also still leaves more affected dentin layer than mechanical techniques using bur. It can be concluded that the use of papain gel and Papacarie® has the same effect of leaving the dentin-affected layer when compared to use of the bur. This study is consistent with several previous studies suggesting that removal of infected dentin with papain gel or Papacarie® is preferable, in accordance with the principle of minimal preparation, because the preparation of caries tissue using chemo-mechanical materials can still leave the affected dentin layer.

4. Conclusion

Based on this research, it can be concluded that dentin-affected micro-hardness after removal of infected dentin with a bur is higher than after removal with papain gel or Papacarie® application. The dentin-treated micro-hardness after removal of infected dentin with Papacarie® was similar to the micro-harness after papain gel application. Removal of infected dentin using Papacarie® or papain gel (chemo-mechanical removal) is better because it leaves more affected dentin than removal using a bur tool (mechanical removal). Papain gel can be used as an alternative natural ingredient for chemo-mechanical preparation. It can be made domestically, and cheaply, and is easily available, but further research is needed to make the papain gel clinically useful.

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