CHAPTER 4

THE SURFACE MICROHARDNESS OF HUMAN ENAMEL AND DENTIN ADJACENT TO THE TOOTH-COLORED RESTORATIVE MATERIALS

4.1. Introduction & Literature review

The hardness is an ability of a material which is considered as resistance to a permanent indentation and can be easily to compare. The process of pressing an indenter into the surface under a specific load for a definite time interval and measuring size or depth of the indentation after the known load was used. The known indenter geometry and length of time will lead into the hardness by calculated from the depth or penetrated contact area under the knowledge of indenter shape and force.

The hardness test is divided into two categories; macrohardness (over 1 kg) and microhardness (less than 1 kg) (Boyer 1987). In static indentation tests, a conical or pyramidal indenter is adopted with the applied load and a relationship of load area or the depth of the indentation is then calculated. Microindentation hardness test or in the other word microhardness test has played an important role in the measurement of the near surface properties. It has been recognized as simple, non-destructive in relation to other mechanical testing procedures. The usual unit of hardness is force per unit area of indentation such as $kgmm^{-2}$ or GPa. The general procedure for hardness testing is to apply standardized force at the penetration point through an indenter. The symmetrical shaped residual indentation impression is measured by microscope for its depth, area or width depending on which testing method is applied. With the fixed standardized load, the indentation dimensions are related to hardness value and the hardness is calculated directly from the machine. The indenters commonly used are Brinell, Knoop, Rockwell and Vickers which have different shapes and size. However, the comparison of different materials should be carried by the same hardness system under similar conditions of indenter, time interval and applied force.

The commonly used microindentation hardness tests for teeth are Knoop and Vickers hardness tests. Knoop is a diamond pyramidal indenter which produces a rhomboid shape residual impression and the indentation dimension of the major axis is measured. Length of the diagonal is measured and reported as Knoop hardness number (KHN). The unit for KHN is kg/mm^2 (Boyer 1987). Vickers hardness test uses a 136^odiamond pyramid which produces a square residual indentation impression. The Vickers indenter appears to penetrate deeper than the Knoop at the same load. The resultant diagonals are measured and reported as Vicker hardness number (VHN). The unit for VHN is kg/mm^2

Vickers hardness test is useful when a small area and a hard material are applied. There were some investigators who used the hardness method to detect changes of enamel hardness after fluoride application (Bartlet, Smith and Wilson, 1994; Diaz-Arnold et al., 1995b). However, there was no report of hardness changes of human enamel and dentin in a series of distance adjacent to the restorative materials. Serra and Cury (1992) tried to investigate the demineralization and remineralization of cervical cavities adjacent to the restoration. Knoop hardness test was used in their studies. The result indicated that glass ionomer cement could interfere the progression of artificial caries-like lesion on enamel adjacent to the restoration. Hick (1986a) and Hitabb et al. (1989) also observed the significance of lesion on enamel adjacent to restoration by using polarized light microscope. Forss and Seppa (1990) reported that the outer enamel surface had some prevention of demineralization adjacent to glass ionomer cement. They also found that different commercial products provided various degree of prevention ability. Marinelli et al (1997) compared enamel remineralization effectiveness of a fluoride rinse, fluoridated dentrifice and fluoride releasing restorative materials. They found that glass ionomer cement and fluoridated dentifrice had significantly greater remineralization effect on adjacent caries than control. Moreover the fluoridated rinse had significantly greater remineralization effect on adjacent caries than the others. The hardness test is a simple index to indicate the effectiveness of prevention to demineralize on enamel and dentin. Pereira et al (1998) also studied the microhardness of in vitro caries inhibited and demineralization adjacent to two kinds of glass ionomer cements. They used Fuji II and Fuji II LC to represent the conventional and resin modified glass ionomer cement respectively. They found that the two materials were effective in producing an acid-resistant layer, but the results were still material dependent.

The main objective in this chapter was to evaluate the hardness change of enamel and dentin adjacent to the fluoride releasing restorative materials. The experiment was divided into two parts. The first part was to collect enamel and dentin specimens that possessed similar VHN range in order to investigate hardness changes in the second part. The second part was to evaluate hardness differences of human enamel and dentin adjacent to the filled restorative materials.

4.2. The comparison of surface microhardness of human enamel and dentin between two positions.

The Vickers surface hardness of two positions next to each other were compared at each level of 0.1 mm. The objective of the differences between two positions was to give that possessed similar range of VHN comparing the surface hardness before soaking in the demineralization solution in order to investigate the hardness changes in the second part. Then the specimens were compared VHN in series of 100, 200 and 300 micron for several positions to obtain the next interpretation of the second part.

4.2.1. Material and methods

Population and samples

Twenty specimens of enamel and dentin were prepared from non carious human premolars. The freshly extracted teeth were collected from the patients aged between 12-20 years. The teeth were kept in sterilized deionized water at 4 °C and the experiments were carried out on teeth within 1 month of storage.

Equipment

1. Microhardness tester (Mitutoyo MVK-G3, Mitutoyo, Japan)
2. Incubator 37°C (Memmert, Schwabuch,Germany)
3. Orbital shaker S03 (Stuart Scientific,Great Britain)
4. Low speed saw cutting machine (Isomet 2000, Buchler, Lalubluff, IL,USA)
5. Universal polisher (Struers, Copenhagen, Denmark)
6. Airotor and cylindical diamond bur diameter 1x1 mm

7. Abrasive paper,silicone-carbide paper grit #600,# 1000 and #2000

Material

Resin composite (Clearfil APX, with Clearfil liner bond 2, Kuraray, Osaka, Japan)

Methods

1.Separation of crown and root portions

The teeth were horizontally cut at the cemento-enamel junction to obtain separate portions of crown and root. The crown portion was prepared for enamel experiment and the root was prepared for the dentin experiment.

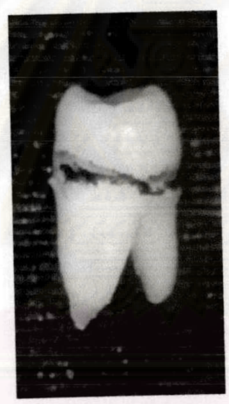


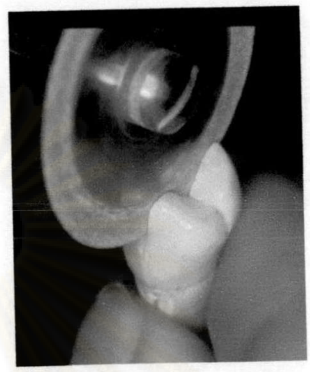
Figure 4.1. The human premolar was separated at horizontal outline to obtain crown and root portions.

2. Preparation for enamel and dentin specimens

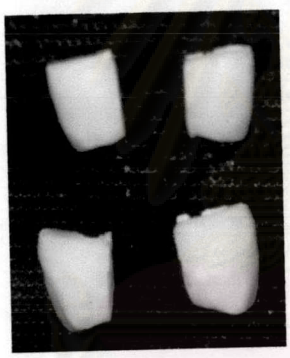
The crown was longitudinally cut to separate buccal and lingual portions. Another longitudinal cut was done on the two cut tooth specimens to obtain two identical halves. One tooth therefore provided 4 test specimens for enamel test. The root was also cut in the same manner and provided 4 dentin specimens (Figure 4.2).

The enamel and dentin specimens were then separately embedded in clear resin facing the enamel and root dentin surface up. Then the enamel and dentin surface were polished with the abrasive paper grit #600, #1000 and #2000 respectively. A final polish was carried out with alumina oxide powder (0.05 micron) with automatic polishing machine until enamel and dentin surfaces were smooth and shinny (Figure 4.3).

A



B

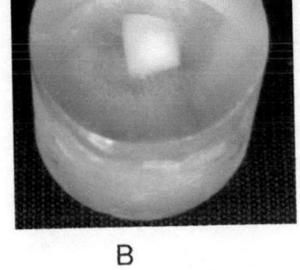


C

D

Figure 4.2 A,B,C and D. The tooth was longitudinally and horizontal cut to obtain 4 enamel and dentin specimens.

A



B

Figure 4.3 A and B. The separately embedded specimens in clear resin

3. Specimens selection

All enamel specimens were selected under stereomicroscope (40x) to discard enamel sample which showed dentin exposure and also dentin specimen which was covered by cementum.

4. Filling with restorative materials

All the twenty enamel and dentin specimens were filled with resin composite (Clearfil APX, Kuraray, Osaka, Japan with Clearfil liner bond 2, Kuraray, Osaka, Japan).

5. Surface hardness measurement

Surface hardness measurements of the prepared enamel and dentin specimens were carried out by Vickers microhardness test in order to compare the differences between the two points.

For enamel, 100 g force was used with duration of 15 seconds. The first series of 6 indentations was performed around the restoration as shown in Figure 4.4. Another second series was made 0.1 mm apart from the first series. The third series was made 0.2 mm from the first series. The total of 18 indentations were made (Figure 4.4).

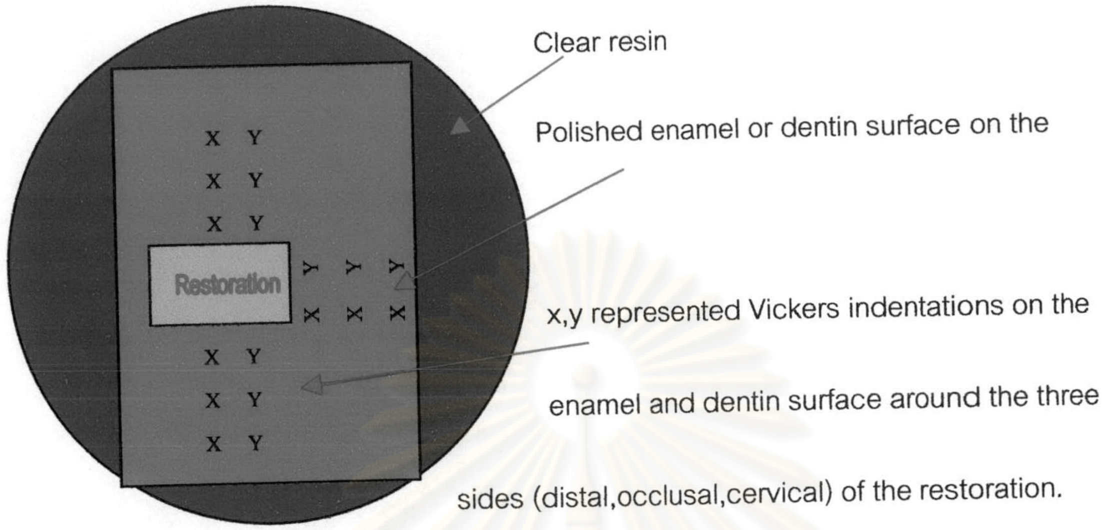


Figure 4.4. Schematic of Vickers surface hardness indentation on enamel and dentin surface

For dentin, 50 g force was used with duration of 15 seconds. Eighteen indentations were made on each specimen following the same pattern created on enamel specimens.

Selection of specimens after measurement

The enamel specimens that gave hardness numbers within the range of 230 - 350 VHN were selected.

The dentin specimens that gave hardness numbers within the range of 30 - 70 VHN were selected.

Statistical Analysis

The enamel and dentin hardness of two points on the same side and at the same distance from the restoration were compared using Paired-samples T test. The significant difference was set at $p<0.05$. The enamel and dentin hardness of the three distances from the restoration were also compared using one way ANOVA. The significant difference was set at $p<0.05$.

4.2.2. Results

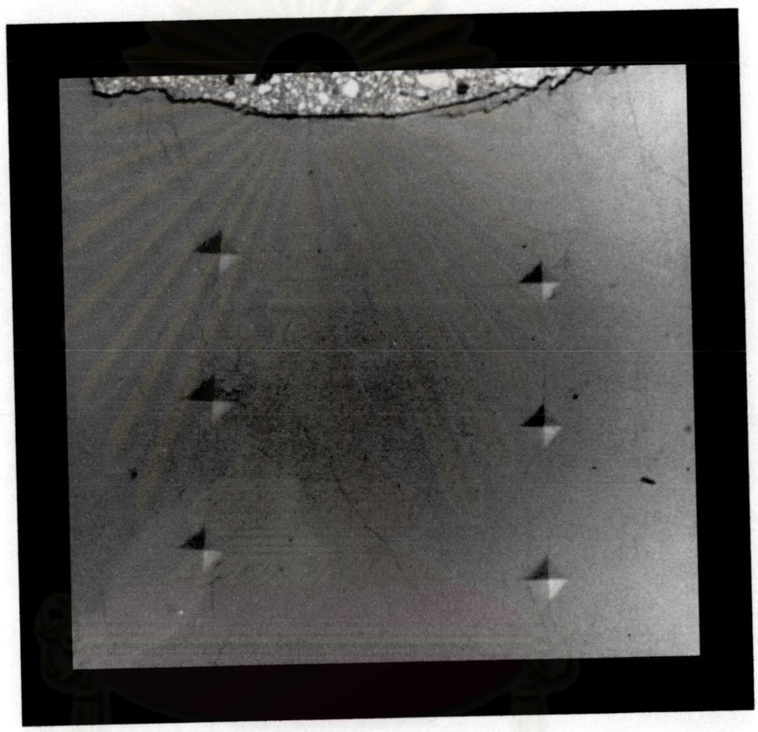


Figure 4.5. Scanning electron micrograph showed similar size of Vickers indentations made around the restoration (200x).

4.2.2.1 Enamel Results

There was no significant difference of enamel hardness between the positions located on the same side and at the same distance from the restoration ($p<0.05$). There was also no significant difference of enamel hardness of the three series of indentations made at three different distance from the restoration ($p<0.05$). Details of statistical analysis were shown in appendix 4.1.

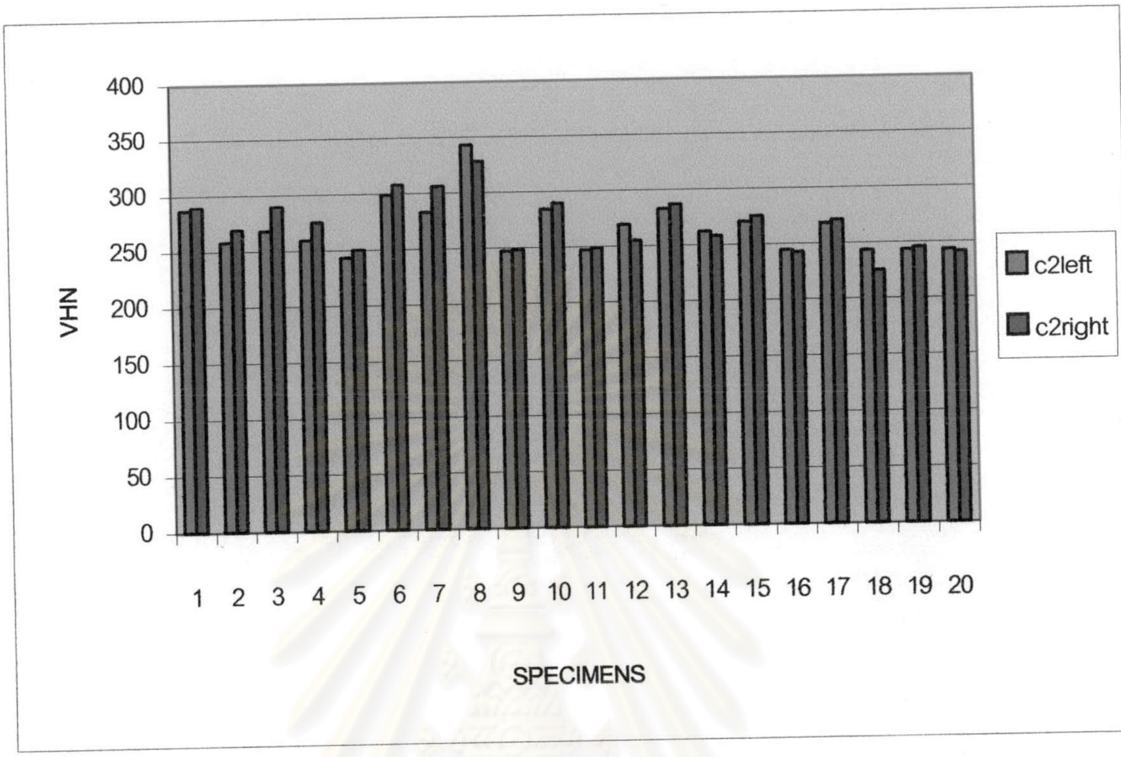


Figure 4.6. Pairs of bar graph represented enamel VHN of two positions next to each other on the cervical side and 0.2 mm from the restoration (20 specimens). The pairs of enamel hardness of all locations were similar to this graph as the data in appendix 4.1.

4.2.2.2 Dentin results

There was no significant difference of dentin hardness between the positions located on the same side and at the same distance from the restoration ($p<0.05$). There was also no significant difference of enamel hardness of the three series of indentations made at three different distances from the restoration ($p<0.05$). Details of statistical analysis were shown in appendix 4.2.

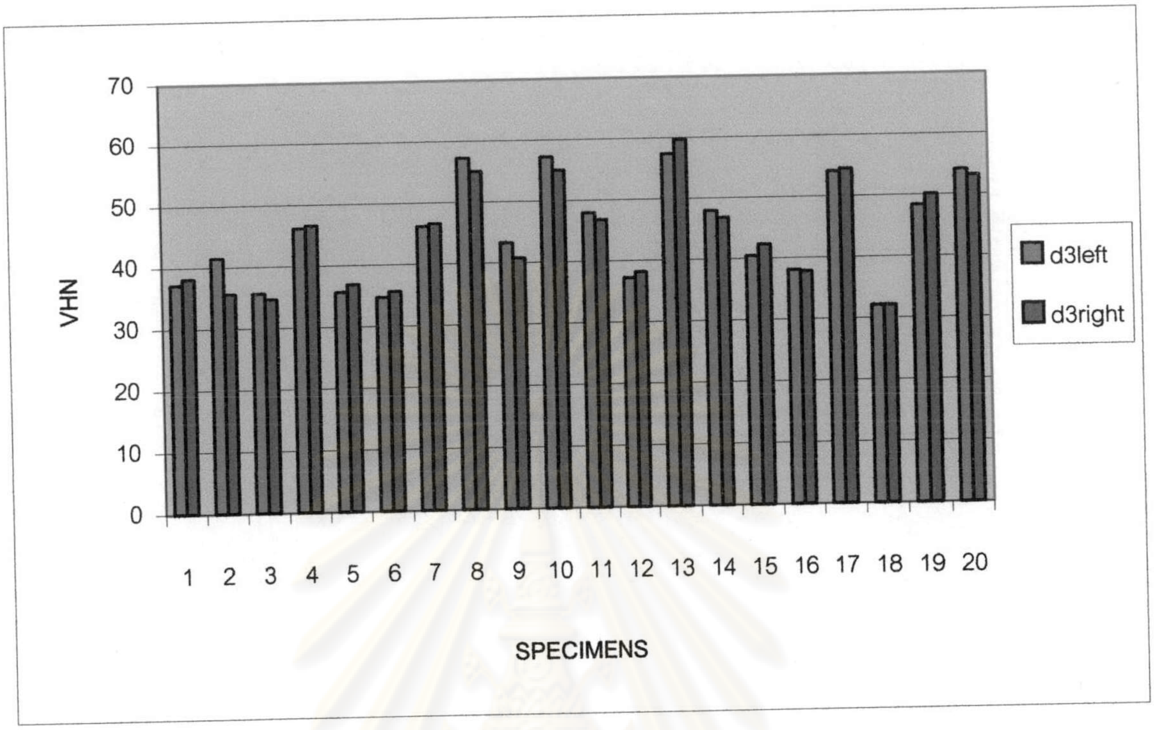


Figure 4.7. Pairs of bar graphs represented the dentin VHN of 2 positions on the distal side 0.3 mm. from the restoration. The pairs of dentin hardness of all locations were similar to this graph as the data in appendix 4.2.

Discussion

The hardness measurement which is non destructive, cheap and easy to carry out can be appropriate method to determine the mechanical properties of materials where there is limited area for the experiment (Waters, 1980; Boyer, 1987). This is because multiple measurements are possible in very limited area such as the enamel and dentin surfaces. The procedure consists of the pressing of the indenter into the surface under the specific load for a definite time interval and the measurement of the size or depth of indentations as the applied load is known. Then the hardness is calculated from the size or depth of the indentations.

This preliminary test was performed to prove the assumption that the indentation hardness of the two positions next to each other were equal. The results showed no statistically significant difference of enamel and dentin microhardness between the two positions next to each other on the measured surface. Therefore, in the next experiment, the hardness measurement before and after artificial caries formation can be done next to each other at the same distance from the material since both positions were proved to give silmilar hardness in normal condition.

The hardness of the three distances 100, 200, 300 microns from the material were shown to be similar (ANOVA). In the next experiment, if there was any change in the hardness as a function of distance, it would not be effect from tooth structure property difference. Then the microhardness difference before and after acid challenge could statistical compared for test groups and the effect on the distance in three series could be compared.

4.3. The microhardness test of enamel and dentin surface adjacent to different restorative materials

4.3.1.Materials & Methods

Population and samples

The enamel and dentin specimens were prepared from non-carious permanent premolar teeth. The freshly extracted teeth were collected from the patient aged between 12-20 years. The teeth was kept in sterilized deionized water (4 °C) and the experiments were carried out on teeth within 1 month of storage.

Equipment

As described in 4.2.1.

Materials

1. Demineralization solution pH 5.0 consisted of

   -Lactic acid 0.1 M

   -Carbopol (C701,B.F. Goodrich company, USA)

   -Hydroxyapatite 50% (Biorad, Laboratory grade, USA)

2. Resin composite (Clearfil APX, Clearfil liner bond 2, Kuraray, Osaka, Japan)
3. Resin modified glass ionomer (Fuji II LC, GC company, Tokyo, Japan)
4. Polyacid modified composite resin or compomer (F2000, 3M company, Minnesota, USA)
5. Sterile deionized water
6. Standard fluoride 10 ppm

Methods

1. Preparation of enamel and dentin specimens

   Following the procedure described in 4.2.1.

2. Selection of enamel and dentin specimens

   Following the procedure described in 4.2.1.

3. Restoration procedure on the selection specimens

All the one hundred and twenty of enamel and dentin specimens were divided into four groups as described.

Group I Resin composite (Clearfil APX, Kuraray, Osaka, Japan with Clearfil liner bond 2, Kuraray, Osaka, Japan) as negative control

Group II Resin composite (Clearfil APX, Kuraray, Osaka, Japan with Clearfil liner bond 2, Kuraray, Osaka, Japan) as positive control

Group III Resin modified glass ionomer cement (Fuji II LC, GC, Tokyo, Japan)

Group IV Polyacid modified composite resin (F2000, 3M company, Minnesota, USA)

A cavity was prepared to the size of 3x3x1 mm in the middle of each enamel and dentin specimens.

For enamel specimens, altogether 60 restorations of negative and positive control groups were filled with resin composite and polished following the manufacturer's instruction. For experimental groups, 30 restorations were made on the enamel specimens using resin modified glass ionomer cement and another 30 restorations using polyacid modified resin composite. The procedures were carried out following the manufacturer's instructions.

For dentin specimens, 120 restorations were made and polished following the manufacturer's instructions using the same materials as done on enamel.

4. Surface hardness measurement before

Surface hardness measurements of all the restored enamel and dentin specimens were measured by Vickers microhardness test.

For enamel, the hardness measurement was performed using 100 g force with duration of 15 seconds. The first series of 9 indentations were performed around 0.1 mm. From the edge of the restoration, three indentations equally apart from each other on three sides (distal, occlusal, cervical) of the restoration (Figure 4.8). Another second series was made 0.1 mm apart from the first series and in the same manner. The third series was also made in the same manner 0.2 mm from the first series. The total of 27 indentations were made as shown in Figure 4.8.

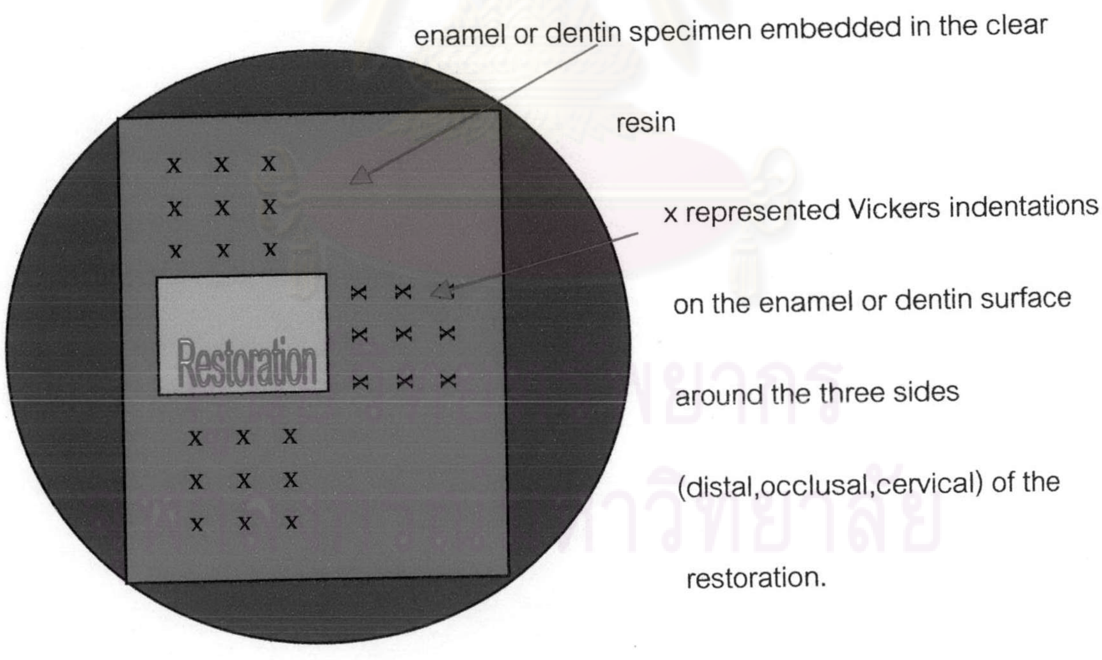


Figure 4.8. Schematic Vickers microhardness measurements before soaking in the demineralization solution

For dentin, the hardness measurement was performed using 50 g force with duration of 15 seconds. Twenty seven indentations were made on each specimen following the same pattern created on enamel specimens.

Selection of specimens for acid challenge experiment

The enamel specimens that gave hardness numbers out of the range of 230 - 350 VHN were excluded.

The denin specimens that gave hardness numbers out of the range of 30 - 70 VHN were also excluded.

With the proposed criteria, 116 enamel and 108 dentin specimens were within the range given. After the selection, the selected specimens were divided into 4 groups.

Group I Composite resin (Clearfil APX, Kuraray, Osaka, Japan with Clearfil liner bond 2, Kuraray, Osaka, Japan) as negative control

Nineteen enamel and seventeen dentin specimens were separately stored in 10 ml demineralization solution in plastic bottles at 37° C for 24 hours and another ten specimens of each were stored for 72 hours.

Group II Composite resin stored with fluoride (Clearfil APX, Kuraray, Osaka, Japan with Clearfil liner bond 2, Kuraray, Osaka, Japan) as positive control

Nineteen enamel and seventeen dentin specimens were separately stored in 10 ml demineralization solution with standard fluoride 10 ppm in plastic bottles at 37° C for 24 hours and another ten samples of each were stored for 72 hours.

Group III Resin modified glass ionomer cement (Fuji II LC, GC, Tokyo, Japan)

Nineteen enamel and seventeen dentin were separately stored in 10 ml demineralization solution in plastic bottles at 37° C for 24 hours and another ten samples of each were stored for 72 hours.

Group IV Polyacid modified composite resin or Compomer (F2000, 3M company, Minnesota, USA)

Nineteen enamel and seventeen dentin specimens were separately stored in 10 ml demineralization solution in plastic bottles at 37° C for 24 hours and another ten samples of each were stored for 72 hours.

All the specimens were shaken on the orbital shaker while stored in the demineralization solution.

5. Surface hardness measurements of the restored specimens after artificial caries induction on enamel and dentin

After 24 and 72 hours, the surface hardness measurements were performed by Vickers microhardness tester (Mitutoyo MVK-G3, Mitutoyo company, Japan) on the enamel and dentin specimens.

For enamel, the hardness measurements were performed using force of 100 g with duration of 15 seconds. For the 24 hours storage specimens, 27 indentations were alternatively made among group of indentations carried out following the previous measurement as shown in figure 4.9. The same force was used for the specimens which stored for 72 hours but only 9 indentations were made among the previous indentations as shown in figure 4.10.

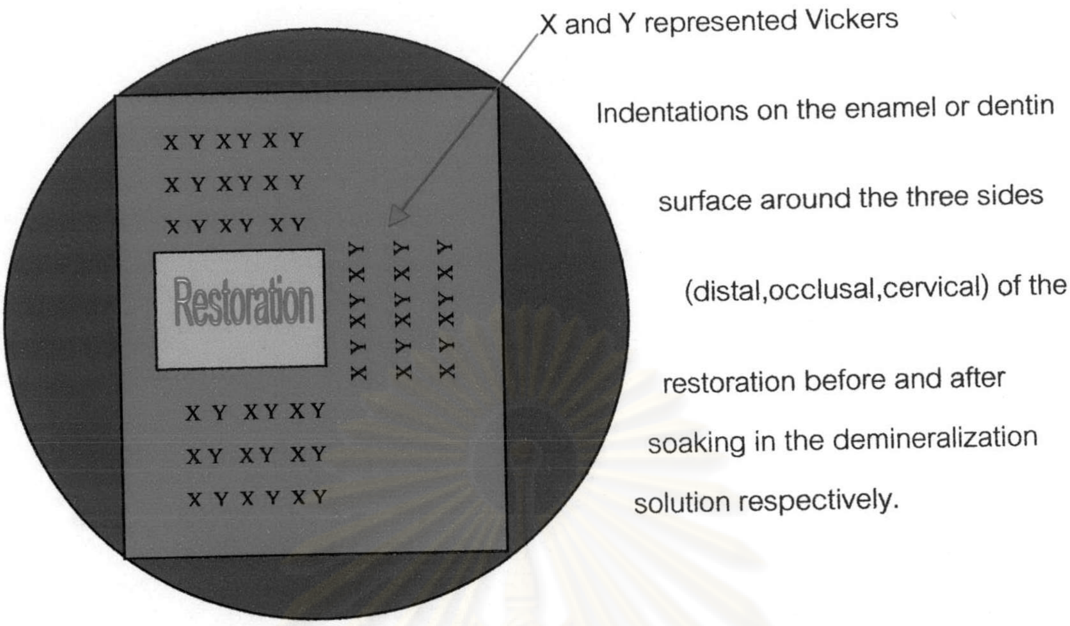


Figure 4.9. Schematic Vickers hardness measurements of enamel and dentin after soaking in the demineralization solution for 24 hours.

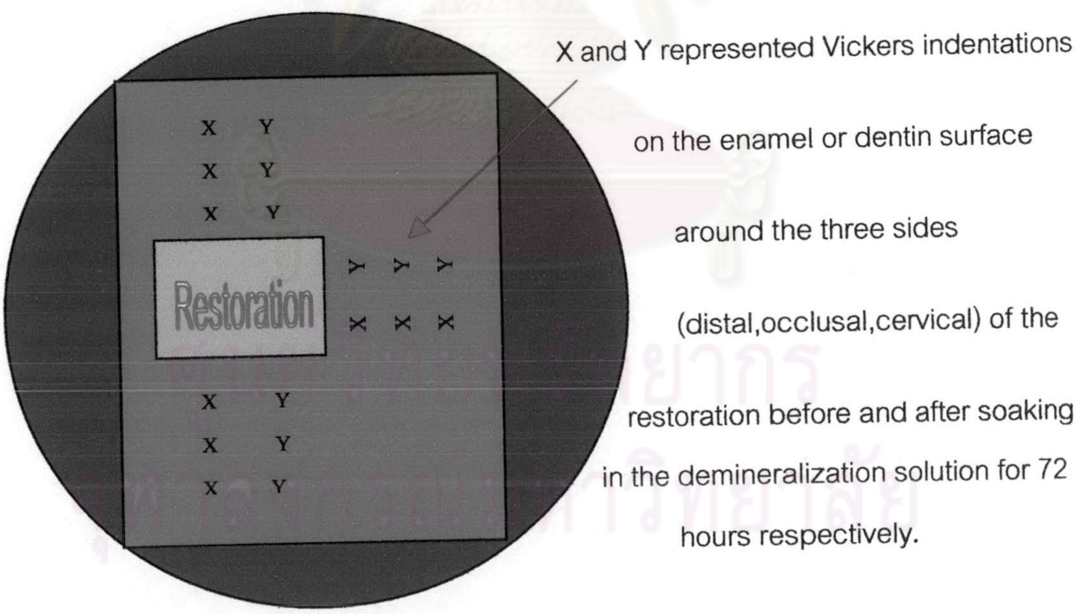


Figure 4.10. Schematic Vickers hardness measurements before and after soaking in the demineralization solution for 72 hours.

For dentin, the hardness measurements were carried out using force of 50 g with duration of 15 seconds. For 24 hours storage specimens, 27 indentations were alternatively made among group of indentations carried out following the previous measurement as shown in figure 4.9. The same force was used for the specimens which stored for 72 hours but only 9 indentations were made among the previous indentations in the same pattern as done on enamel specimens (Figure 4.10).

Then the surface hardness measurements were carried out to find hardness differences within series and between the series.

Statistical analysis was carried out by one way ANOVA at $p<0.05$ followed by Bonferroni for Post Hoc test.

4.3.2. Results

The results were exhibited in two categories of enamel and dentin.

4.3.2.1 Changes of enamel microhardness after soaking in the demineralization solution for 24 hours

The results of the enamel hardness changes after soaking in the demineralization solution for 24 hours for the four groups studied were exhibited in Table 4.1. The changes of the surface hardness were presented in terms of delta and comparison of the mean delta was determined using one way ANOVA and Bonferroni for Post Hoc tests. The result showed significant difference among the mean delta of the four groups as shown in figure 4.11. The mean delta of negative control group was 182.03 ± 54.37 VHN which showed the greatest among all groups. The positive group showed the least changes of 33.58 ± 30.04 VHN. The mean delta of the surface microhardness of resin modified glass ionomer cement and polyacid modified resin composite were 69.37 ± 40.03 and 111.74 ± 56.81,respectively. They were in between the negative and positive groups.

Table 4.1. The mean delta and standard deviation of the enamel hardness changes after soaking in the demineralization solution for 24 hours among the four groups studied.

	N	Mean of delta	Standard Deviation
Group I	459	182.03	54.37
Group II	459	33.58	30.04
Group III	459	69.37	40.03
Group IV	458	111.74	56.81

Note: There was statistically significant difference among the four groups studied.

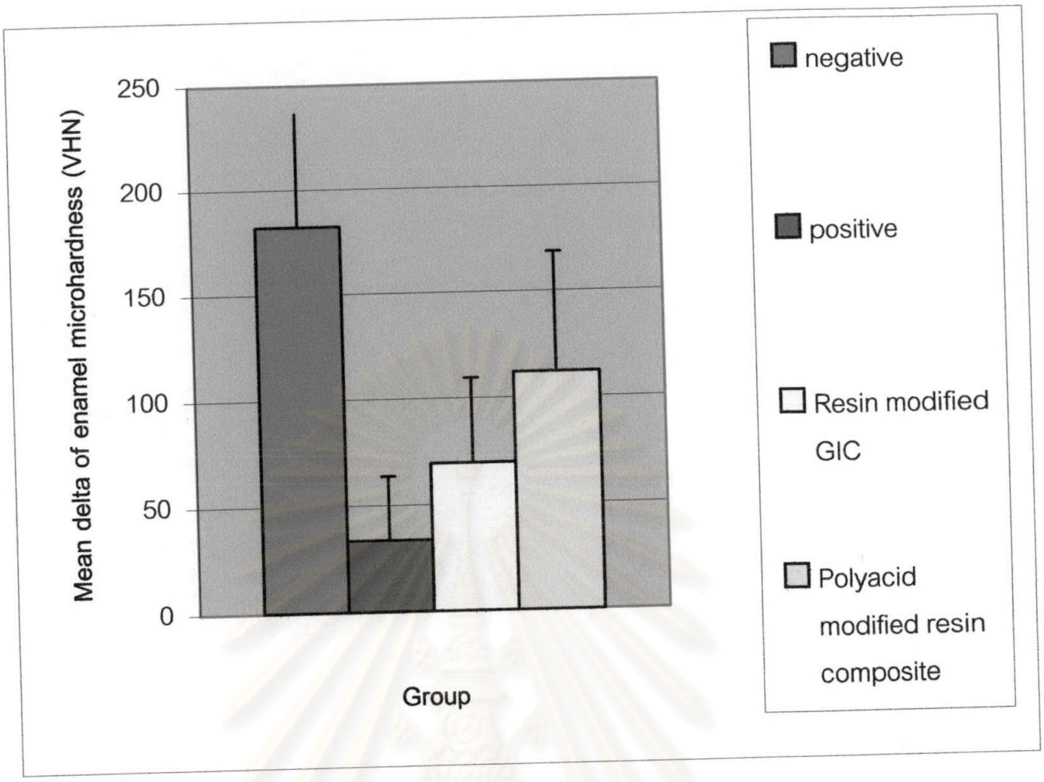


Figure 4.11. Bar graph indicated the mean delta of all the surface hardness changes of the four groups studied after soaking in the demineralization solution for 24 hours.

The hardness changes of enamel as a function of distance from the restoration (100 , 200 and 300 micron) after soaking in the demineralization solution for 24 hours were demonstrated in Table 4.2-4.5 and Figure 4.12. The results of delta hardness of negative and positive control groups exhibited no significant difference among the three distances studied (ANOVA,$p<0.05$).

For the resin modified glass ionomer cement, the mean delta of 200 and 300 micron groups showed higher significantly different than that of 100 micron group (Table 4.4 , Figure 4.12).

For the polyacid modified resin composite, the mean delta of 300 micron groups exhibited higher significantly different than that of 100 micron group (Table 4.4 , Figure 4.12).

Table 4.2. The mean delta and standard deviation of hardness measured before and after soaking enamel specimens group I in the demineralization solution for 24 hours.

Distance from the restoration	N	Mean delta	Standard Deviation
100 microns	153	183.29 *	53.96
200 microns	153	183.62 *	53.46
300 microns	153	179.18 *	55.87

Note : The same affix showed no significant difference among group (p<0.05).

Table 4.3. The mean delta and standard deviation of hardness measured before and after soaking enamel specimens group II in the demineralization solution for 24 hours.

Distance from the restoration	N	Mean delta	Standard Deviation
100 microns	153	31.29 *	29.22
200 microns	153	32.78 *	28.45
300 microns	153	36.67 *	32.26

Note : The same affix showed no significant difference among group (p<0.05).

Table 4.4. The mean delta and standard deviation of hardness measured before and after soaking enamel specimens group III in the demineralization solution for 24 hours.

Distance from the restoration	N	Mean delta	Standard Deviation
100 microns	153	58.94	40.95
200 microns	153	72.45 *	39.44
300 microns	153	76.73 *	37.72

Note : The same affix showed no significant difference among group (p<0.05).

Table 4.5. The mean delta and standard deviation of hardness measured before and after soaking enamel specimens group IV in the demineralization solution for 24 hours.

Distance from the restoration	N	Mean	Standard. Deviation
100 microns	153	102.90 *	56.76
200 microns	153	113.69 *, **	56.01
300 microns	152	118.68 **	56.86

Note : The same affix showed no significant difference among group ($p<0.05$).

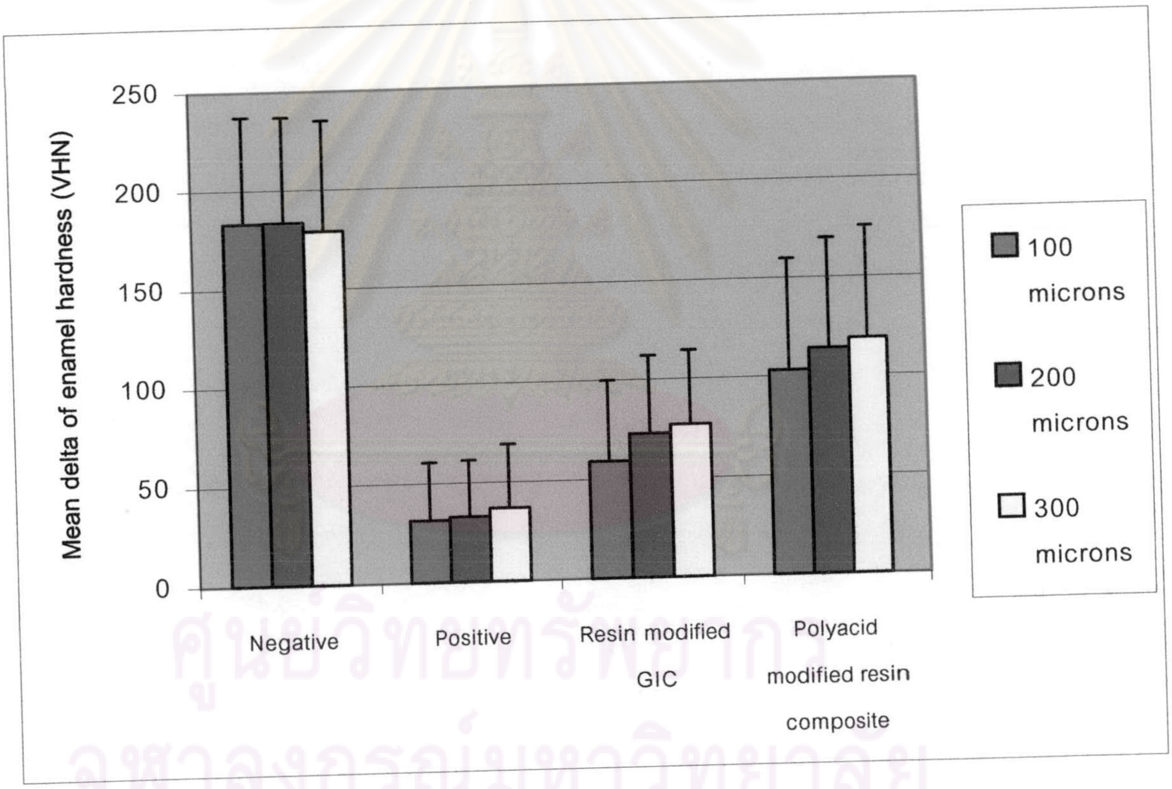


Figure 4.12. Bar graphs showed changes of mean delta of enamel hardness as a function of distance after soaking in the demineralization solution for 24 hours for each group of material.

4.3.2.2. Changes of enamel microhardness after soaking in the demineralization solution for 72 hours

The mean difference of the surface microhardness before and after soaking in the demineralization solution for 72 hours showed similar results to those soaking for 24 hours. The mean delta was also determined using one way ANOVA and Bonferroni for Post Hoc tests $p<0.05$). The result exhibited significant difference among the mean delta of the four groups studied (Figure 4.13).

For the negative control group, the mean delta showed the highest value of 200.87 ± 47.49 VHN, while the positive control group gave the least mean delta of 35.20 ± 27.35 VHN. The mean delta of the two fluoride releasing materials groups was in between the negative and positive control groups. The resin modified glass ionomer showed less change than polyacid modified resin composite group (Table 4.6).

Table 4.6. The mean delta and standard deviation of the enamel hardness changes after soaking in the demineralization solution for 72 hours among the four groups studied.

	N	Mean delta	Standard Deviation
Group I	135	200.87	47.49
Group II	134	35.20	27.35
Group III	135	70.65	43.93
Group IV	134	96.06	51.55

Note: There was statistically significant difference among the four groups studied.

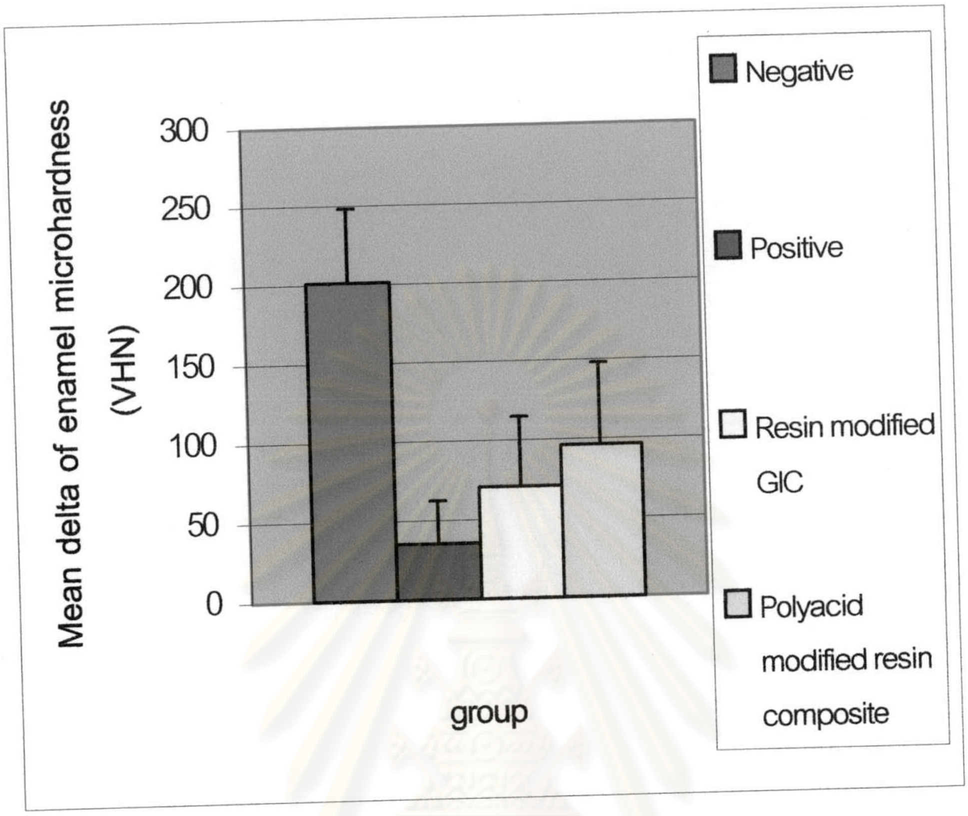


Figure 4.13. Bar graph indicated the mean delta of all the surface hardness changes of the four groups studied after soaking in the demineralization solution for 72 hours.

The hardness changes of enamel as a function of distance from the restoration (100 , 200 and 300 microns) after soaking in the demineralization solution for 72 hours were demonstrated in Table 4.7-4.10 and Figure 4.14. The results of delta hardness of negative and positive control groups exhibited no significant difference among the three distances studied (ANOVA, $p<0.05$).

For the resin modified glass ionomer cement, the mean delta of 200 and 300 microns groups showed higher significantly different than that of 100 micron group (Table 4.9 , Figure 4.14).

For the polyacid modified resin composite, the mean delta of 300 microns groups exhibited higher significantly different than that of 100 microns group (Table 4.10, Figure 4.14).

Table 4.7. The mean delta and standard deviation of hardness measured before and after soaking enamel specimens group I in the demineralization solution for 72 hours.

Distance from the restoration	N	Mean	Standard Deviation
100 microns	45	200.09 *	49.54
200 microns	45	199.35 *	48.19
300 microns	45	203.18 *	45.65

Note : The same affix showed no significant difference among group ($p<0.05$).

Table 4.8. The mean delta and standard deviation of hardness measured before and after soaking enamel specimens group II in the demineralization solution for 72 hours.

Distance from the restoration	N	Mean	Standard Deviation
100 microns	44	36.07 *	28.28
200 microns	45	34.53 *	25.69
300 microns	45	35.02 *	28.59

Note : The same affix showed no significant difference among group ($p<0.05$).

Table 4.9. The mean delta and standard deviation of hardness measured before and after soaking enamel specimens group III in the demineralization solution for 72 hours.

Distance from the restoration	N	Mean	Standard Deviation
100 microns	45	55.89	38.41
200 microns	45	77.99 *	44.81
200 microns	45	77.99 *	44.81
300 microns	45	78.06 *	45.38
300 microns	45	78.06 *	45.38

Note : The same affix showed no significant difference among group ($p<0.05$).

Table 4.10. The mean delta and standard deviation of hardness measured before and after soaking enamel specimens group IV in the demineralization solution for 72 hours.

Distance from the restoration	N	Mean	Standard Deviation
100 microns	45	80.76 *	49.44
200 microns	44	100.69 *, **	50.82
300 microns	45	106.84 **	51.80

Note : The same affix showed no significant difference among group (p<0.05).

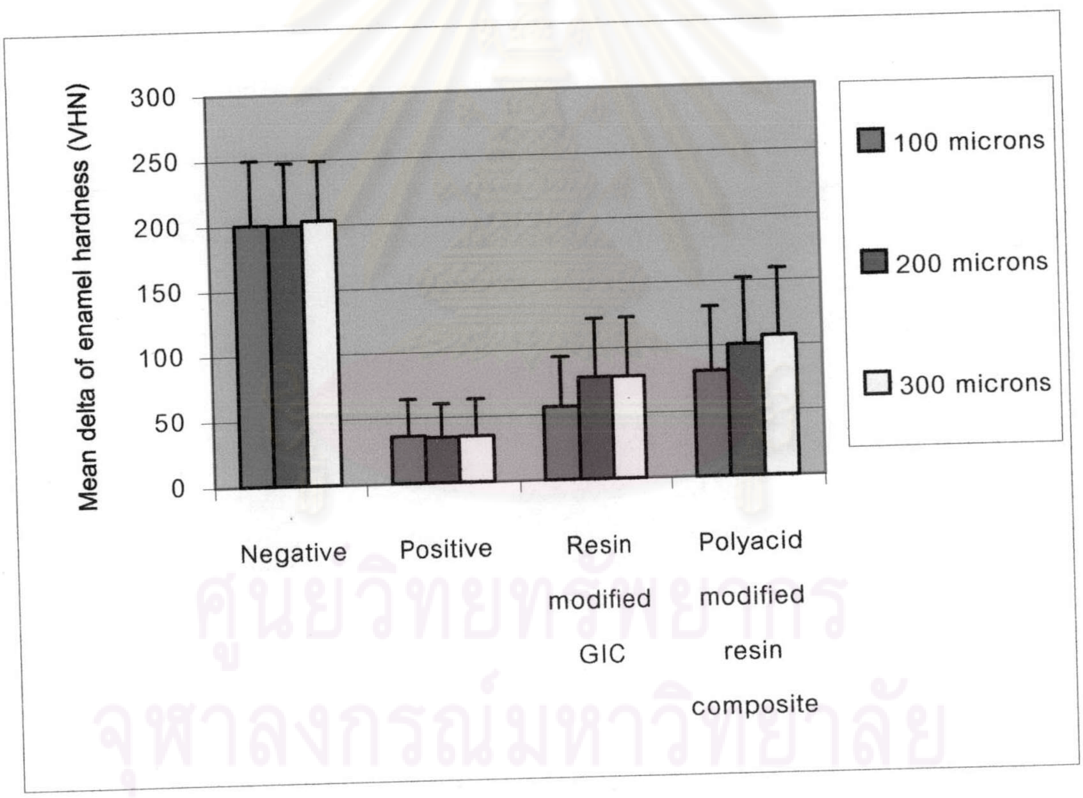


Figure 4.14. Bar graphs demonstrated enamel hardness changes as a function of distance after soaking in the demineralization solution for 72 hours for the four groups studied.

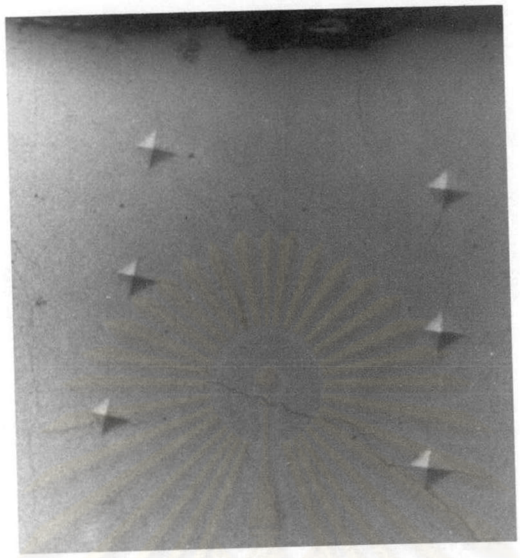


Figure 4.15. Scanning electron micrograph showed the difference in size of Vickers indentation impressions changes in group II (100x), the right impressions were made after soaking in the demineralization solution for 72 hours.

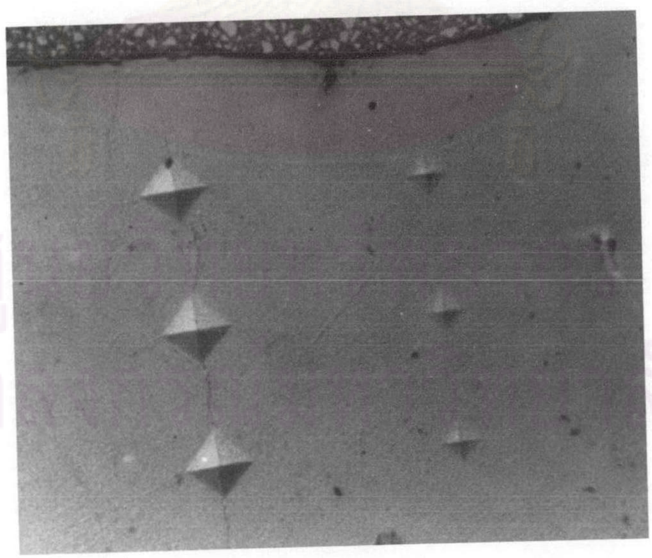


Figure 4.16. Scanning electron micrograph showed the difference in size of Vickers indentation impressions changes in group IV (100x), the larger impressions were made after soaking in the demineralization solution for 72 hours.

4.3.2.3. Changes of dentin microhardness after soaking in the demineralization solution for 24 hours

The results of dentin hardness changes after soaking in the demineralization solution for 24 hours were demonstrated for the four groups in Table 4.11 and Figure 4.17. The mean difference of the microhardness change in group I showed the largest (25.63 ± 8.07 VHN) while the least was found in group II (18.08 ± 8.55 VHN). The statistical comparison was analyzed by one way ANOVA at $p<0.05$. The results shown that there was statistically significant difference between the four test groups. Bonferroni as Post Hoc tests revealed that negative control group was statistically significant difference from the others and so did positive group. There were no significant difference between group III (resin modified glass ionomer cement) and group IV (Polyacid modified resin composite).

Table 4.11. The mean delta and standard deviation of the dentin hardness changes after soaking in the demineralization solution for 24 hours among the four groups studied.

	N	Mean delta	Standard Deviation
Group I	432	25.63	8.07
Group II	432	18.08	8.55
Group III	432	22.33 *	11.50
Group IV	432	23.63 *	9.88

Note : The same affix showed no significant difference between groups.

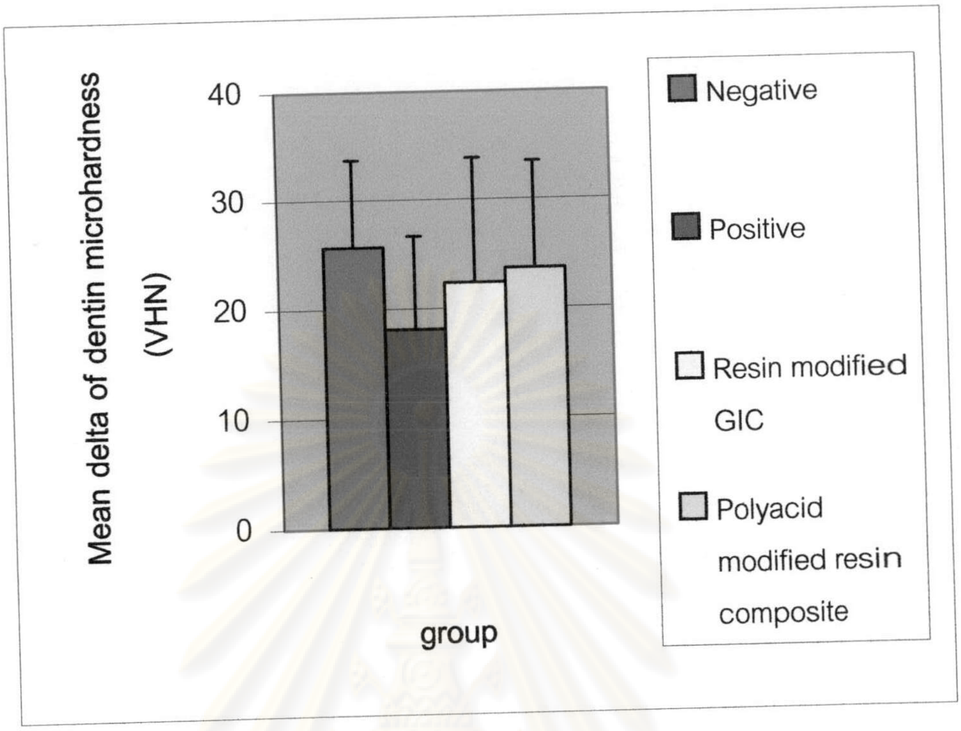


Figure 4.17. Bar graph indicated the mean delta of surface dentin microhardness of the four groups studied after soaking in the demineralization solution for 24 hours.

The hardness changes of dentin as a function of distance from the restoration (100 , 200 and 300 microns) after soaking in the demineralization solution for 24 hours were demonstrated in table 4.12 - 4.15. The results of delta hardness of negative and positive control groups exhibited no significant difference among the three distances studied (ANOVA,$p<0.05$).

For the resin modified glass ionomer cement, the mean delta of 300 microns groups showed higher significantly different than that of 100 microns group (Figure 4.18).

For the polyacid modified resin composite, the mean delta of 300 microns groups exhibited higher significantly different than that of 100 microns group (Figure 4.18).

Table 4.12. The mean delta and standard deviation of hardness measured before and after soaking dentin specimens group I in the demineralization solution for 24 hours

Distance from the restoration	N	Mean delta	Standard Deviation
100 microns	144	25.46 *	7.75
200 microns	144	25.79 *	8.51
300 microns	144	25.64 *	7.98

Note : The same affix showed no significant difference among groups.

Table 4.13. The mean delta and standard deviation of hardness measured before and after soaking dentin specimens group II in the demineralization solution for 24 hours

Distance from the restoration	N	Mean	Standard Deviation
100 microns	144	18.55 *	9.11
200 microns	144	17.84 *	8.31
300 microns	144	17.84 *	8.26

Note : The same affix showed no significant difference among groups.

Table 4.14. The mean delta and standard deviation of hardness measured before and after soaking dentin specimens group III in the demineralization solution for 24 hours

Distance from the restoration	N	Mean	Standard Deviation
100 microns	144	19.98 *	11.15
200 microns	144	22.91 *,**	11.88
300 microns	144	24.10 **	11.13

Note : The same affix showed no significant difference among groups.

Table 4.15. The mean delta and standard deviation of hardness measured before and after soaking dentin specimens group IV in the demineralization solution for 24 hours.

Distance from the restoration	N	Mean delta	Standard Deviation
100 microns	144	22.23 *	9.82
200 microns	144	23.29 *,**	9.52
300 microns	144	25.37 *,**	10.10

Note : The same affix showed no significant difference among groups.

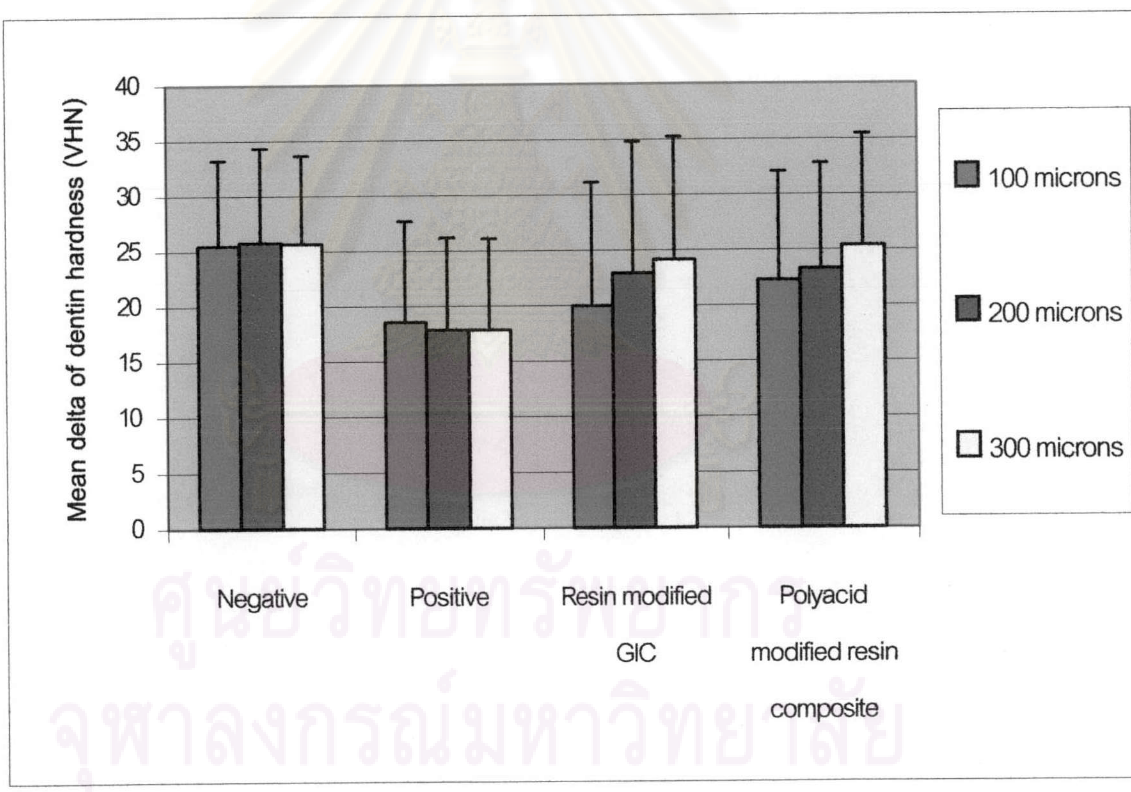


Figure 4.18. Bar graph showed changes of mean delta of dentin hardness as a function of distance after soaking in the demineralization solution for 24 hours.

4.3.2.4. Changes of dentin microhardness after soaking in the demineralization solution for 72 hours

The mean difference of the surface microhardness before and after soaking in the demineralization solution for 72 hours was showed in Table 4.16. There was statistically significant difference of the mean data among the groups studied (ANOVA, $p<0.05$). For the negative control group, the mean delta showed the highest value of 32.11 ± 5.70 VHN, while the positive control group gave the least mean delta of 25.96 ± 6.51 VHN. The mean delta of the two fluoride releasing materials groups were in between the negative and positive control groups. The resin modified glass ionomer showed less change than polyacid modified resin composite group (Table 4.16 and Figure 4.19).

Table 4.16 The mean delta and standard deviation of the dentin hardness changes after soaking in the demineralization solution for 72 hours among the four groups studied.

	N	Mean	Standard Deviation
Group I	135	32.11 *	5.70
Group II	135	25.96	6.51
Group III	135	28.46	4.86
Group IV	135	30.70 *	5.64

Note : The same affix showed no significant difference among groups.

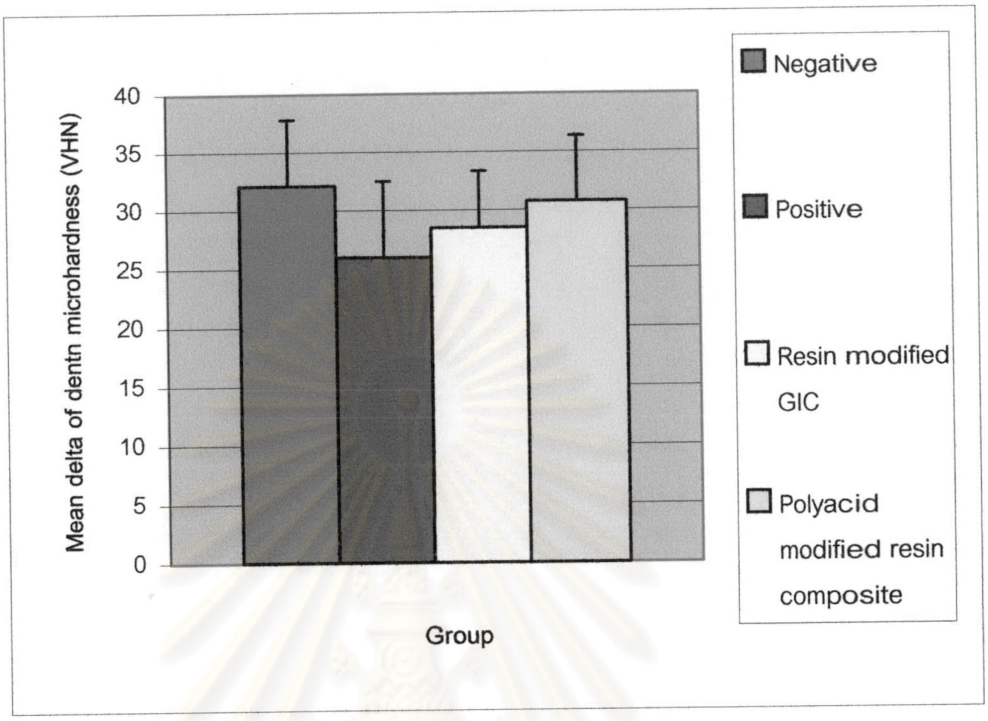


Figure 4.19. Bar graph indicated the mean delta of all the surface microhardness changes of the four groups studied after soaking in the demineralization solution for 72 hours.

The hardness changes of dentin as a function of distance after soaking in the demineralization solution for 72 hours were demonstrated in Table 4.17-4.20 and Figure 4.20. The results of delta difference showed no significant difference among the distance in group I and II (Table 4.17-4.18). The mean delta of dentin hardness at 100 microns distance was significantly lower than those of 300 microns from the interface in both resin modified glass ionomer cement and polyacid modified resin composite (Table 4.19-4.20).

Table 4.17. The mean delta and standard deviation of hardness measured before and after soaking dentin specimens group I in the demineralization solution for 72 hours.

Distance from the restoration	N	Mean delta	Standard Deviation
100 microns	45	32.29 *	6.04
200 microns	45	32.38 *	5.53
300 microns	45	31.66 *	5.61

Note : The same affix showed no significant difference among groups.

Table 4.18. The mean delta and standard deviation of hardness measured before and after soaking dentin specimens group II in the demineralization solution for 72 hours.

Distance from the restoration	N	Mean delta	Standard. Deviation
100 microns	45	25.52 *	7.26
200 microns	45	25.97 *	6.36
300 microns	45	26.40 *	5.98

Note : The same affix showed no significant difference among groups.

Table 4.19. The mean delta and standard deviation of hardness measured before and after soaking dentin specimens group III in the demineralization solution for 72 hours.

Distance from the restoration	N	Mean delta	Standard. Deviation
100 microns	45	26.88	4.45
200 microns	45	29.14 *	4.84
300 microns	45	29.37 *	4.99

Note : The same affix showed no significant difference among groups.

Table 4.20. The mean delta and standard deviation of hardness measured before and after soaking dentin specimens group IV in the demineralization solution for 72 hours.

Distance from the restoration	N	Mean	Standard Deviation
100 microns	45	28.8111	5.5180
200 microns	45	31.5867 *	5.4478
300 microns	45	31.7027 *	5.5749

Note : The same affix showed no significant difference among groups.

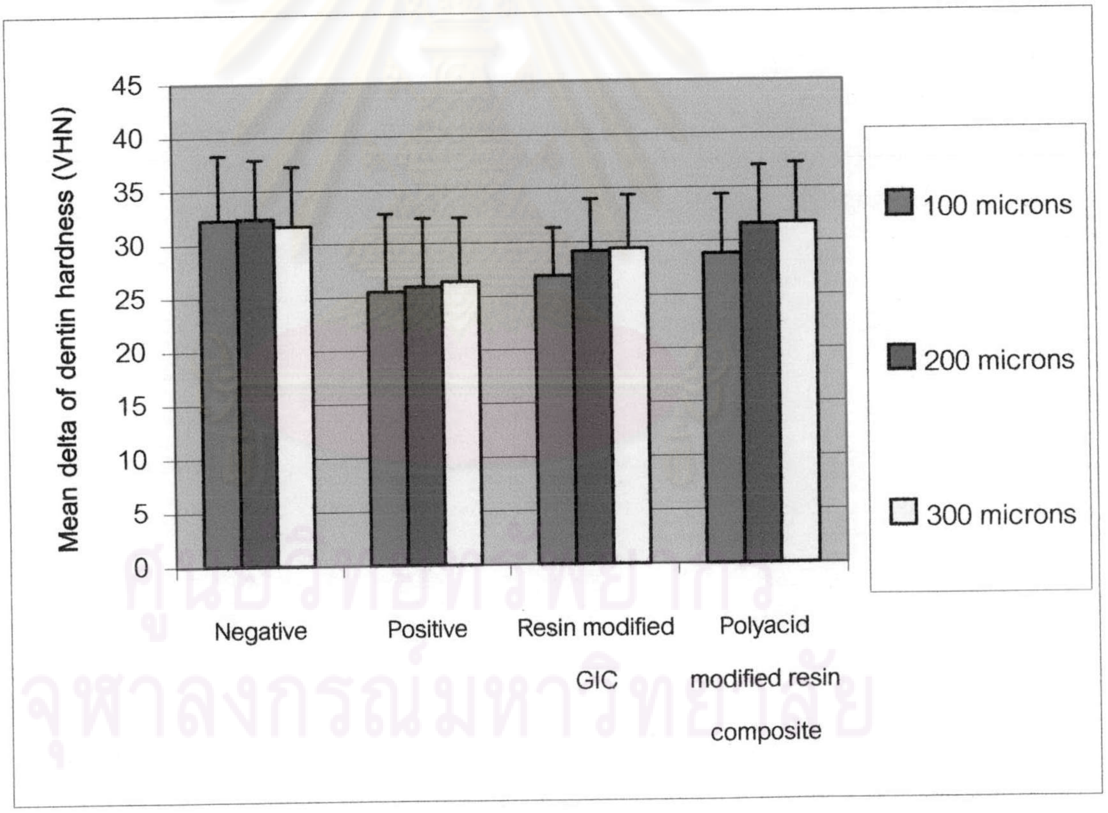


Figure 4.20. Bar graph indicated changes of mean delta of dentin hardness as a function of distance after soaking dentin specimens in the demineralization solution for 72 hours.

4.3.3. Discussion

Non carious human premolars were used in the experiments in order to investigate the surface hardness changes of human teeth after acid challenges. Most investigators tried to avoid human teeth because the size of human teeth was difficult to manipulate. The most popular enamel and dentin specimens used in the experiment were bovine samples (Arends and van der Zee,1990b; Attin et al.,1997; Tantbirojn et al.,1997). However, the human premolars would represent the actual surface hardness.

The cutting design was made in order to compare surface hardness measurement before and after soaking in the demineralization solution using the same tooth. This comparison was accomplished in order to give the real information of human surface hardness change of enamel and dentin from human teeth.

The surface measurements were attempted and the specimens were selected before they were soaked in the demineralization solution. The artificial caries lesion was produced by the demineralization solution (pH5) which permitted consistent formation of outer lesions (White,1987b;1995). The surface hardness measurements of the same specimens were repeated on the positions next to the first measurement (Figure 4.9) after soaking in the demineralization solution. Since the two positions next to each other were proved to have no significant difference hardness, the indentation made after soaking in the demineralization solution could be determined to have measured on the same spot.

After deminerization process, the surface hardness of tooth structure reduced due to loss of mineral content under acidic condition. Considerate investigators have been carried out the demineralization, remineralization and fluoride uptake by enamel and dentin. Fluoride was proved to be able to reduce the rate of demineralization and enhance crystallite growth (Arends and Christofferson,1986; ten Cate, Damen and Buijs, 1998). When demineralization occurs hydroxyapatite losts its hydroxy ion and fluoride ion in the surrounding environment replaces and forms to be fluoroapatite which is harder (ten Cate and Loveren,1999). If fluoride had effect on the tooth surface, the microhardness which is an simple index should be detected (Waters,1980; Boyer,1987). However, the surface microhardness changes as a function of distance in human enamel and dentin affected from fluoride releasing material have not been reported.

Many investigators determined the effect of fluoride in reducing secondary caries (Arends, Schuthof and Jongebloed, 1979;1980b; Forsten, 1995; Tam, Chan and Yim,1997). They all agreed that fluoride releasing materials had caries inhibition effect in vitro under polarized light microscope. The process of caries inhibition was the effect from fluoride releasing material. However, the hardness of tooth surface which can represent the index of mechanical strength after using fluoride releasing materials has not been clarified in terms of the distance from the restoration. Some previous reports suggested that glass ionomer cement may promote hypermineralization of carious lesions by possibly depositing minerals and fluoride, therefore acid resistance increased (ten Cate and van Duinen, 1995; Modesto et al.,1997).

In this study there were some mechanical property changes in enamel group with resin modified glass ionomer cement restoration within the distance of 100 μm to 300 μm from the edge of the materials. At the same time there were no enamel hardness changes during the three distances in control groups. Due to this result the inhibition zone affected by resin modified glass ionomer cement and polyacid modified resin composite was in a limiting area not more than 100 μm. Pereira et al. (1998) studied in vitro caries formation and measured the width of inhibition zones under polarized light microscope. They found a distinct inhibition zone adjacent to both conventional and resin-modified glass ionomer cements. And the width of inhibition zone was also in the area of 100 μm which agreed with the result of this study.

The potential to increase tooth resistance to secondary caries is due to the type of glass ionomer cement , initial fluoride content , mixing procedure , setting time and also pH change in environment (Thornton et al., 1986b; Sidhu and Watson, 1995; Forss and Seppa, 1990). The surface microhardness of human enamel and dentin in this study can be implied that the fluoride had effects on the surface hardness changes and also agreed that type of materials had effects in prevention of secondary caries since there were statistically significant difference among groups of materials. Featherstone et al. (1990) suggested that 1 ppm of fluoride in acid solution reduced the dissolution rate of carbonate apatite in vitro. This report was confirmed by the present study that the positive group which contained fluoride 10 ppm showed a little surface hardness change compared to other groups after soaking in the demineralization solution.

Fluoride was thought to be the major caries inhibitor by inhibiting demineralization, enhancing remineralization and inhibiting bacteria activity. The caries process begins when acid diffuses through the porous enamel or exposed dentin, dissociating to produce hydrogen ions. The hydrogen ions readily dissolve the mineral, freeing calcium and phosphate from the tooth structure surface into solution. The major effects of fluoride through out the body occurs in mineralization tissues. Biological hydroxyapatite may dissolve in acidic environment and free $(OH)^-$. If $(OH)^-$ in hydroxyapatite is replaced by fluoride ion, it turns to be fluorapatite which is more acid resistance. Both resin modified glass ionomer cement and polyacid modified resin composite released fluoride and showed some differences of hardness change among distances studied while the negative and positive control groups showed none. This also supported that surface hardness change adjacent to the fluoride releasing materials within 100 microns was less than the other two distances studied. This can be implied that enamel 100 microns from the edge of the restoration tended to be harder.

The causes can be explained in terms of fluoride which can speed up the remineralization process by adsorbing to the surface and attracting calcium ion. The newly fluorapatite formed so that the crystal would behave like low solubility when soaked in acid solution.

In previous study, it was found that there was some amounts of fluoride released from the restorative materials. Further study is required to correlate the amount of fluoride and hardness in different areas. This would confirm the influence of fluoride on tooth surface hardness. This chapter studied only at the surface of the enamel and dentin adjacent to the restoration. The influence of fluoride releasing material on tooth structure as a function in depth is also required. This would be investigated in the following chapter.