CHAPTER 5

SUBSURFACE NANOHARDNESS OF HUMAN ENAMEL AND DENTIN ADJACENT TO THE TOOTH-COLORED RESTORATIVE MATERIALS

5.1. Introduction & Literature review

Microhardness test has a limitation when used in small area because its size and depth of penetration are in micrometer scale even when the smallest forces of 1 N was applied. Moreover, small force produces small impression which is difficult to visualize in the optical measurement, and hence operator bias is inevitable and an inaccurate result is possibly obtained (Van Meerbeek et al., 1993; Willems, 1993a). The new generation of ultra microindentation or nanoindentation instrument was introduced (Bell et al., 1991/1992). This system was developed for measurement of a near surface properties with nanometer resolution. This technology can control indentation process with initial forces substantially less than 0.1 mN, progressively determining depths of penetration with resolution less than 1 nm and force resolution better than 0.01 mN (Bell et al., 1991/1992).

The hardness is calculated from force and projected area. The operation of the instrument is controlled by a personal computer. The indenter is driven into the surface of a testing material until the resistance reaches the set force. The data are obtained from the penetration on loading and also the elastic recovery of the indentation on the unload procedure. The Berkovich indenter, a diamond pyramid form, is the most common in the determination of hardness because of its great stiffness and hardness of diameter tip. The Berkovich indenter with face angles of 65.3° has projected area equivalent to Vickers indenter at the same depth and thus give the degree of equivalence in results (Bell et al.,1991/1992). The nanohardness test can provide the measurement of near surface of a small area adjacent to the restorative material since the depth is in nanometer scale thus give very small size of indentation impression where microhardness tester cannot perform. This instrument is also completely automatic and high precision.

Nanohardness test is the new method to determine the tooth structure properties. It provides the measurement of near surface which can be a small area adjacent to the restorative material. The hardness of the test material is calculated from the depth of the indentation as well as the load. Consequently, the ability to reproduce the small indentation area using nano loads enables the measurement of very small area.

Willems et al. (1993a) was the first who reported the hardness and Young's modulus of dental restorative materials and enamel by nanoindentation technology. Van Meerbeek et al. (1993) also investigated the resin-dentin interface (hybrid layer) with nanoindentation technique. However, their nanoindentation tester made slightly oversized indentations relative to the hybrid layer. The result could be the properties of mixture of hybrid layer, resin and dentin rather than hybrid layer alone. Pereira et al. (1998) correlated Knoop microhardness and triangular hardness by measuring the microhardness in vitro caries inhibited and demineralized dentin adjacent to the conventional and resin modified glass ionomer cement. Knoop and triangular hardness indentations were performed perpendicular to the surface and parallel to the cavity wall in the demineralized lesion. The Knoop indentations were performed 100 μm from the top and 50 μm intervals parallel to the cavity margin. The triangular hardness indentations were made adjacent to the restorative materials as close as the information provided from the manufacturer. They found that triangular hardness correlated with Knoop hardness. The later technology seems to be an alternative method for measuring the narrow surfaces.

The UMIS nanoindentation test apparatus was developed by CSIRO Inc. (Australia) in 1995. This nanoindentation device has a computer controlled X-Y table and triangular pyramidal diamond indentation point with capacity to develop loads of 0.1 mN – 500 mN. The indentation points are subjected for identification by computer. The capacity to reproduce small nano points with the diamond pyramid enables the small areas of dentin and other tooth structures where the microhardness test method cannot. However, there was not any report of the subsurface hardness of human enamel and dentin adjacent to the restoration. The surface microhardness changes as the function of distance were investigated in the previous chapter. The objective of this chapter was to achieve the subsurface hardness changes as the function of distance of the tooth structure adjacent to the restoration.

5.2. Material & method

Population and samples

The same group of specimens used in chapter 4.

Equipment

1. Ultra-microindentation hardness tester system (UMIS 2000, CSIRO, Sydney, Australia)
2. Low speed saw cutting machine (Isomet 2000, Buehler, Lake Bluff, USA)
3. Polishing machine (Struers, Copenhagen, Denmark) , diamond particle (1 μm) and alumina powder (0.05 μm)
4. Abrasive paper,silicone-carbide paper grit #600, 1000 and 2000
5. Paralleling machine (Leitz, Wetzlar, Germany)
6. wax

Method

The four groups of enamel and dentin specimens were cut cross-sectionally through the restoration shown in figure 5.1.

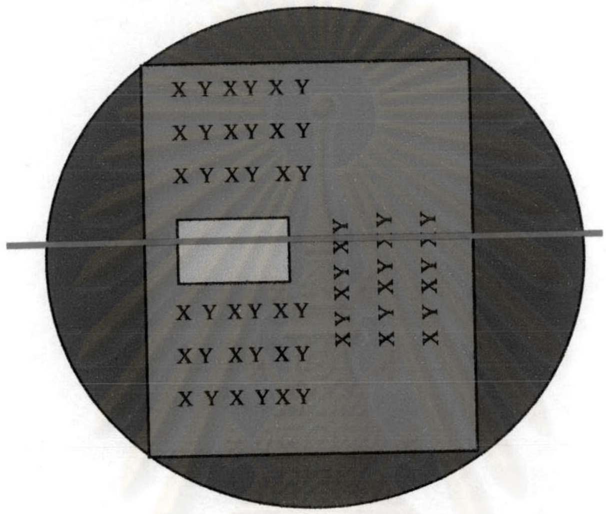

Figure 5.1 Cross-sectional cutting (red line) through the restoration

The internal surfaces of the cut specimens were polished by abrasive paper and polishing machine to a final finish with 0.5 μm alumina oxide. The specimens were mounted on the metal base with wax (Figure 5.2) and using the paralleling machine to make the cut surface parallel to the horizontal plane (Figure 5.3). The area next to the interface between the restored material and tooth structure was measured using UMIS (Figures 5.4-5.5). The indenter used in this study was a diamond tip with an equilateral triangular base of 65.3 ° face angle to the axis. The hardness measurement was carried out by indenting the Berkovich indenter (Synton BA, Switzerland) on the given positions generated by computer. The results were generated by the software program and the maximum depths of the penetration were reported for every indenting positions generated on the specimen surface. From the maximum depth the hardness can be calculated from the following equation.

$$H = P/A$$

Where P is a pressure or force and A is the contact area which given by

$$A = Kh_p^2$$

where h_p is the depth of penetration and K is a geometric constant (for a Berkovich indenter is 24.5) ($K = \sqrt{3}\tan 2\theta$, where $\theta = 65.3^{\circ}$). The results in this chapter were reported in maximum depth (h_p) which was related to the hardness.

Figure 5.2. Mount specimens on the metal stubs

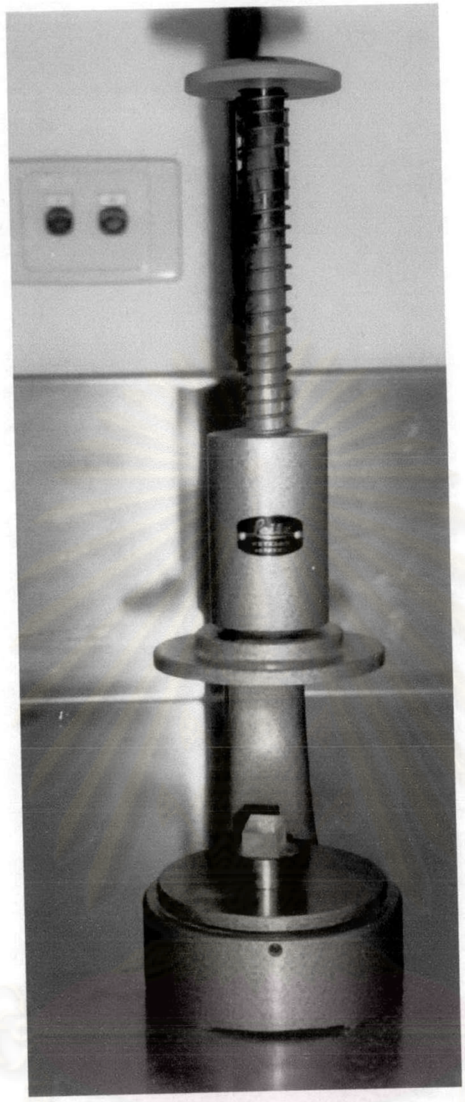

Figure 5.3. Paralleling machine was used to parallel the specimen surface to the stub.

The measurements of penetration depth in series of penetrations indentations were carried out for both enamel and dentin. For each tooth structure, the measurements were performed using two different indentation distances. The first measurement was carried out for the distance of 20 μm and 15 μm for enamel and dentin respectively. The second measurement was carried out for the distance of 50 μm for both enamel and dentin specimens.

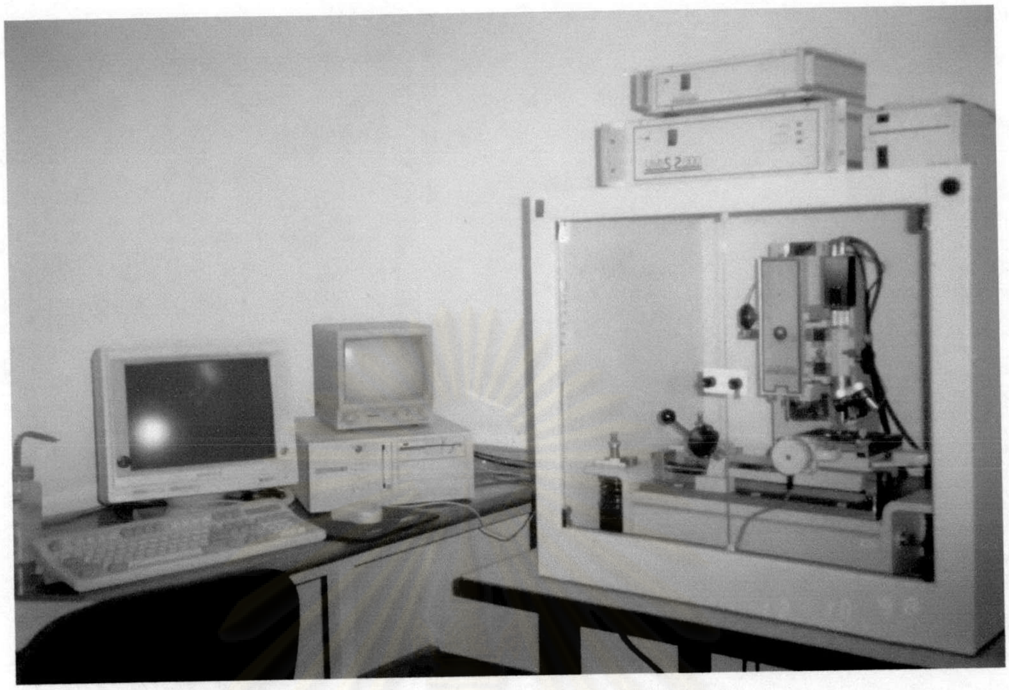

Figure 5.4 Ultra microindentation system (UMIS 2000, CSIRO,Sydney,Australia)

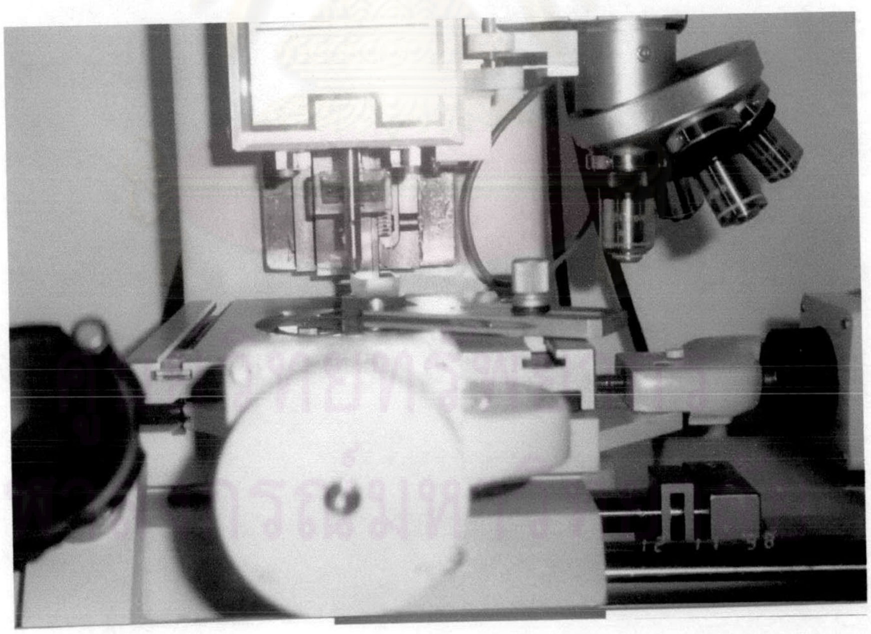

Figure 5.5 The sample was positioned on the stage of the UMIS and subjected to the nanohardness measurement

5.2.1 Measurement of maximum penetration depth on enamel.

5.2.1.1. Indentation spacing distance of 20 μm

Eight indentations were made using a force of 80 mN (8 g) at the area 20 microns (μm) distance from the enamel – material interface. Another eight indentations were made parallel to the interface and with a separate spacing of 20 microns. Another eight indentations were made in the same manner. Altogether 5 series of 8 indentations were made as shown in figure 5.6.

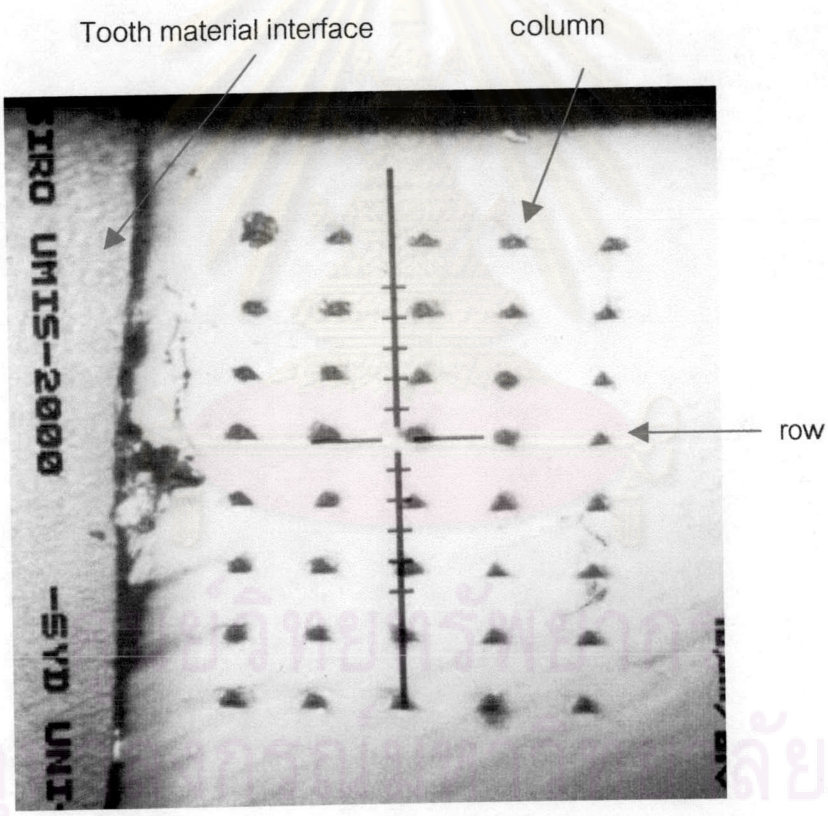

Figure 5.6. Five series of eight indentations were made at every 20 μm from the tooth material interface with the separated spacing of 20 μm (20x).

5.2.1.2. Indentation spacing distance of 50 μm

Four indentations were made using a force of 80 mN (8 g) at a distance of 25 microns (μm) next to the tooth material interface. Another four indentations were made parallel to the interface and with a separate spacing of 50 microns. Another four indentations were made in the same manner. Altogether 6 series of 4 indentations were made as shown in figure 5.7.

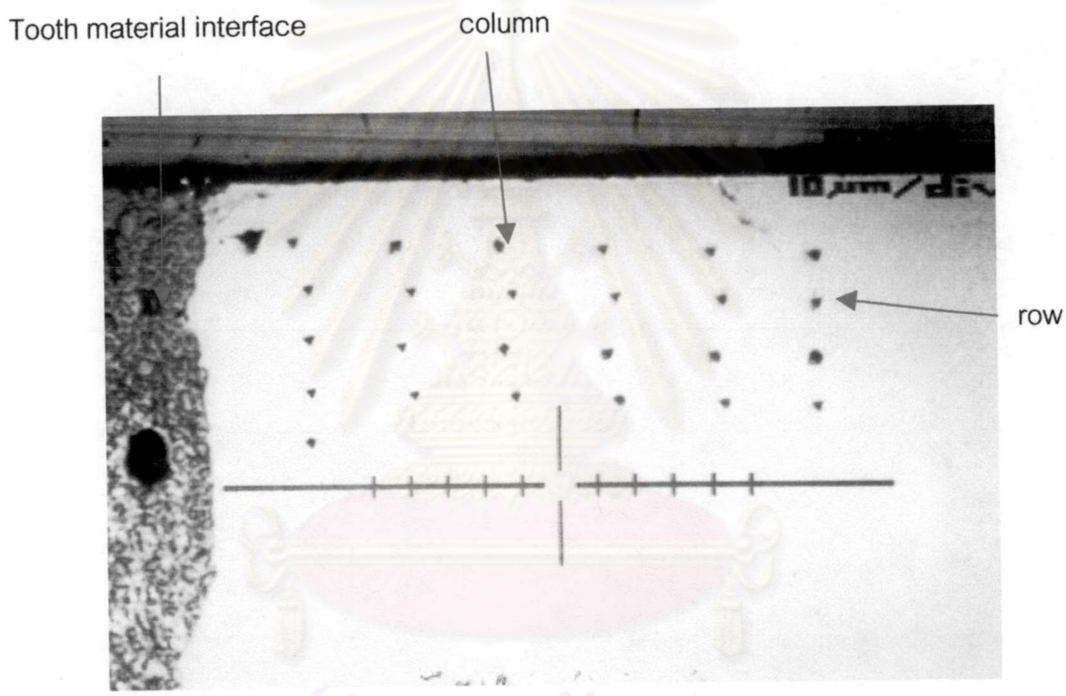

Figure 5.7. Six series of four indentations were made at every 50 μm from the tooth material interface with the separated spacing of 50 μm (10x).

5.2.2. Measurement of maximum penetration depth on dentin.

5.2.2.1. Indentation spacing distance of 15 μm

Eight indentations were made using a force of 20 mN (2 g) at the area 15 microns (μm) distance from the enamel – material interface. Another eight indentations were made parallel to the interface and with a separate spacing of 15 microns. Another eight indentations were made in the same manner. Altogether 5 series of 8 indentations were made as shown in figure 5.8.

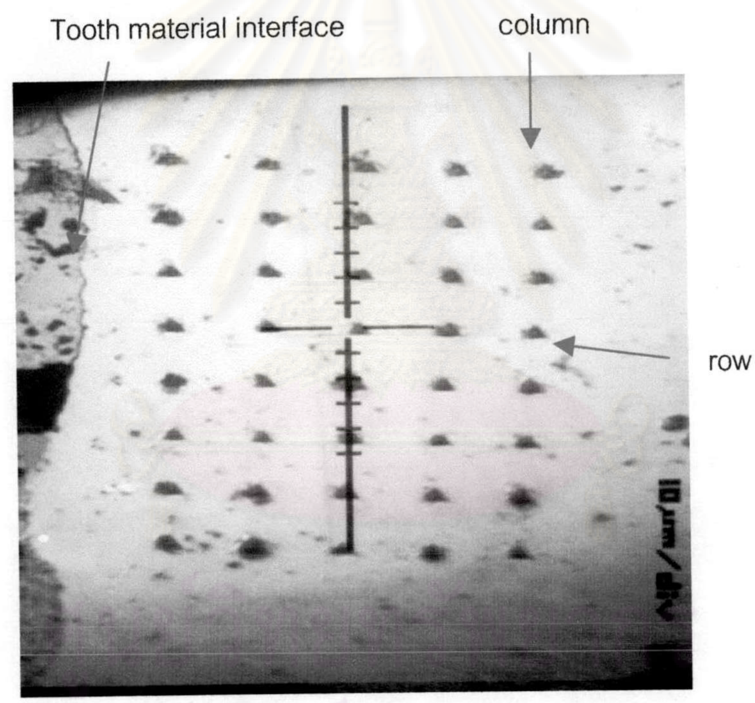

Figure 5.8. Five series of eight indentations were made at every 15 μm from the tooth material interface with the separated spacing of 15 μm (20x)

5.2.2.2. Indentation spacing distance of 50 μm

Four indentations were made using a force of 20 mN (2 g) at a distance of 25 μm microns next to the dentin material interface. Another four indentations were made parallel to the interface and with a separate spacing of 50 microns. Another four indentations were made in the same manner. Altogether 6 series of 4 indentations were made as shown in figure 5.9.

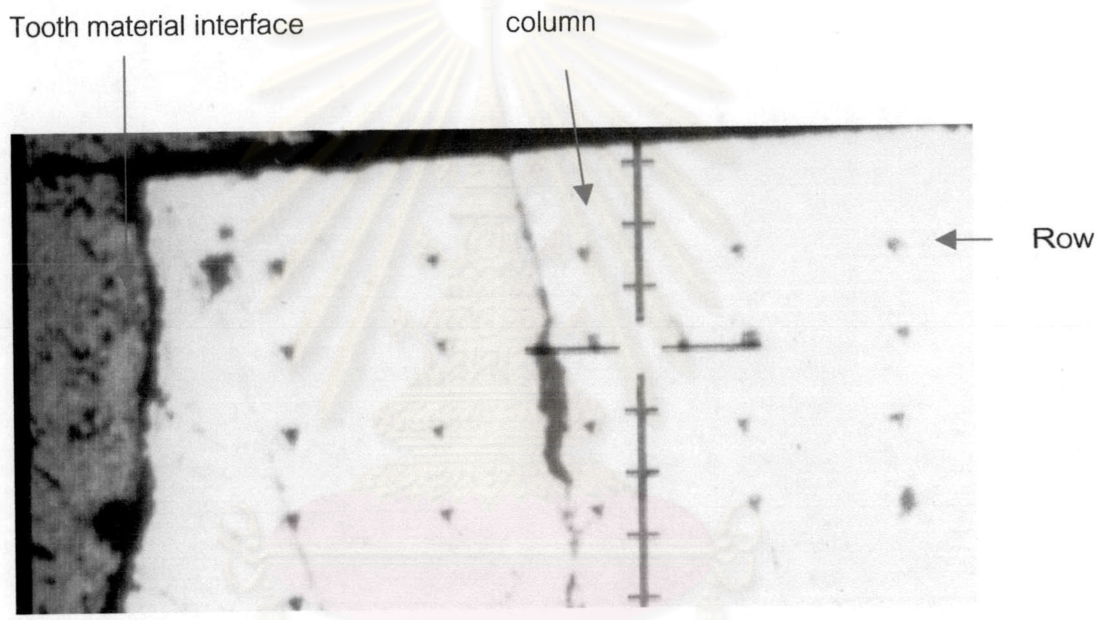

Figure 5.9. . Six series of four indentations were made at every 50 μm from the tooth material interface with the separated spacing of 50 μm (10x).

5.3. Results

5.3.1. Measurement of maximum penetration depth on enamel.

5.3.1.1. Maximum depth of the series made on enamel by indentation spacing distance of 20 μm

The maximum depths of each indentations in the column and row of each group were indicated as a sample in Table 5.1 - 5.4. The negative group showed the deepest indentation depth, while the positive group exhibited the least. The indentation depth of the resin modified glass ionomer cement and polyacid modified resin composite groups were almost the same and their depths were between the negative and positive groups as in shown Figure 5.10. The comparison average of all the average maximum depth for each material in terms of columns and rows were shown in Figures 5.10 - 5.11.

The statistical comparison were determined by column and row of each group. The results showed that there was not any statistically significant between column or row in each group. However, there were statistically difference between the four groups of enamel in the whole area of the investigation using ANOVA and Bonferroni as the Post Hoc tests (Table 5.5 and Figure 5.12).

Table 5.1. The maximum indentation depth in nanometer (nm) of the enamel specimens for the 5 columns and 8 rows of negative group

Negative group		Column				
		1	2	3	4	5
Row	1	1013.63	996.76	983.44	990.92	967.05
	2	969.23	936.23	961.18	943.05	972.91
	3	944.07	930.54	944.56	960.24	962.95
	4	940.53	954.63	922.86	928.01	950.74
	5	953.58	933.60	930.31	943.70	976.49
	6	934.89	959.79	980.80	961.11	932.05
	7	944.74	960.48	937.36	958.81	949.51
	8	939.45	930.48	938.65	932.22	965.10

Table 5.2. The maximum indentation depth in nanometer (nm) of the enamel specimens for the 5 columns and 8 rows of positive group

Positive group		Column				
		1	2	3	4	5
Row	1	886.69	903.47	893.26	898.94	891.77
	2	862.10	861.21	875.81	924.47	863.75
	3	870.82	882.17	861.22	871.23	857.81
	4	874.41	900.70	875.49	898.33	876.99
	5	882.3	861.97	870.80	881.44	896.98
	6	870.10	879.27	872.29	893.78	886.11
	7	872.94	883.88	885.92	885.72	876.31
	8	886.1	881.69	878.35	881.17	883.38

Table 5.3. The maximum indentation depth in nanometer (nm) of the enamel specimens for the 5 columns and 8 rows of resin modified glass ionomer cement group

Resin modified glass ionomer cement		Column				
		1	2	3	4	5
Row	1	955.87	963.27	960.26	919.88	905.07
	2	949.35	974.01	910.98	939.29	919.45
	3	935.92	924.10	901.57	906.81	903.04
	4	916.36	907.56	904.76	915.64	910.86
	5	978.71	949.03	962.21	928.43	913.07
	6	947.04	931.43	953.20	937.28	912.92
	7	975.94	956.66	986.57	966.80	932.69
	8	970.81	958.44	958.09	920.20	929.76

Table 5.4. The maximum indentation depth in nanometer (nm) of the enamel specimens for the 5 columns and 8 rows of polyacid modified resin composite group

Polyacid modified resin composite		Column				
		1	2	3	4	5
Row	1	990.30	991.29	980.17	961.13	983.28
	2	960.68	957.39	944.65	1004.31	916.95
	3	1047.58	931.87	1011.96	937.22	962.59
	4	978.38	909.59	873.44	937.60	930.54
	5	926.67	886.73	924.26	929.30	894.48
	6	935.84	971.10	904.17	919.96	897.39
	7	930.92	889.55	876.18	920.91	879.86
	8	916.18	918.82	896.46	895.23	902.39

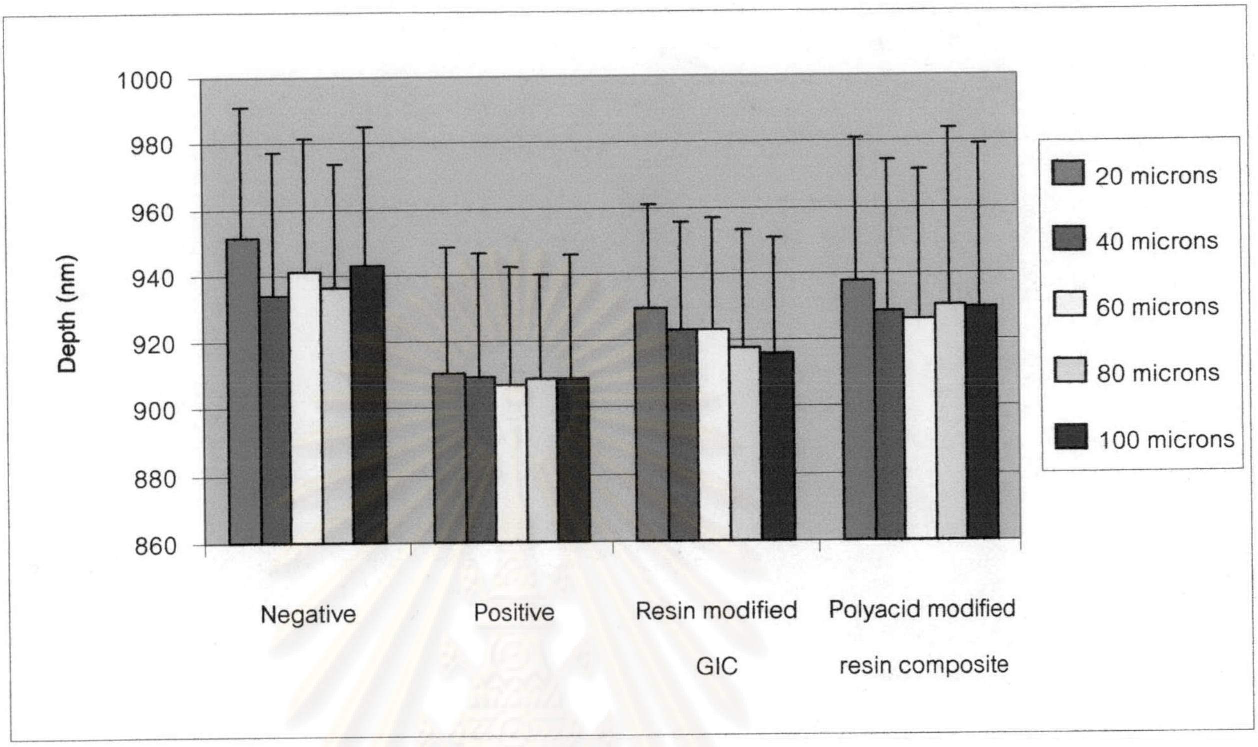

Figure 5.10. Bar graph indicated the subsurface mean penetration depth as a function of distance from the enamel material interface in 5 columns

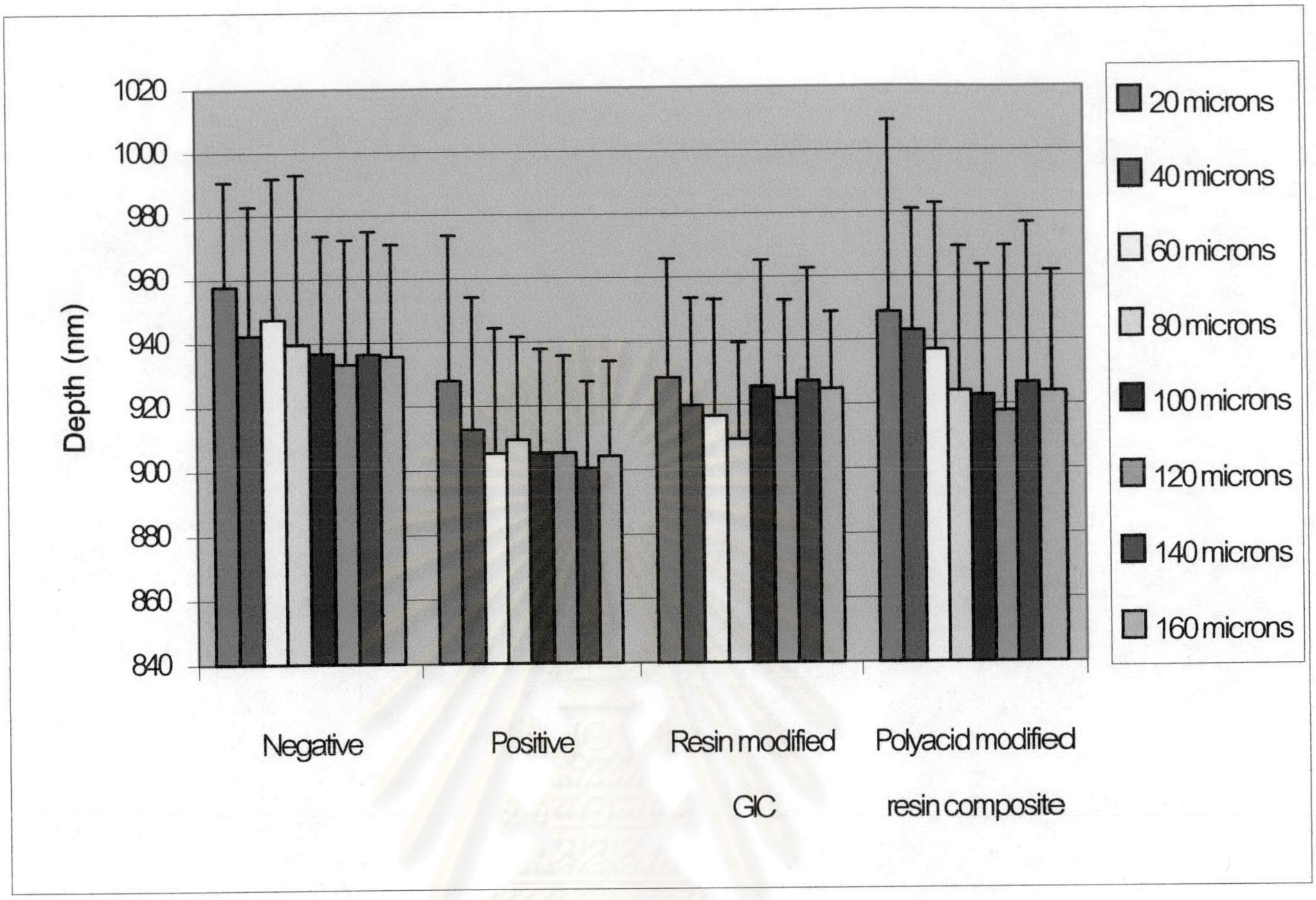

Figure 5.11. Bar graph indicated the subsurface mean penetration depth as a function of distance from enamel surface in 8 rows

Table 5.5. The mean maximum penetration depth of the whole investigation area of all the groups

Group	N	Mean (nm)	Standard Deviation	Standard. Error	Minimum	Maximum
Negative	277	940.99	40.79	2.45	773.72	1093.95
Positive	278	908.86	35.62	2.136	836.66	1011.78
Resin mofied glass ionomer cement	277	921.79 *	33.79	2.03	831.83	1007.64
Polyacid modified resin composite	280	930.41 *	47.46	2.84	831.83	1108.41

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

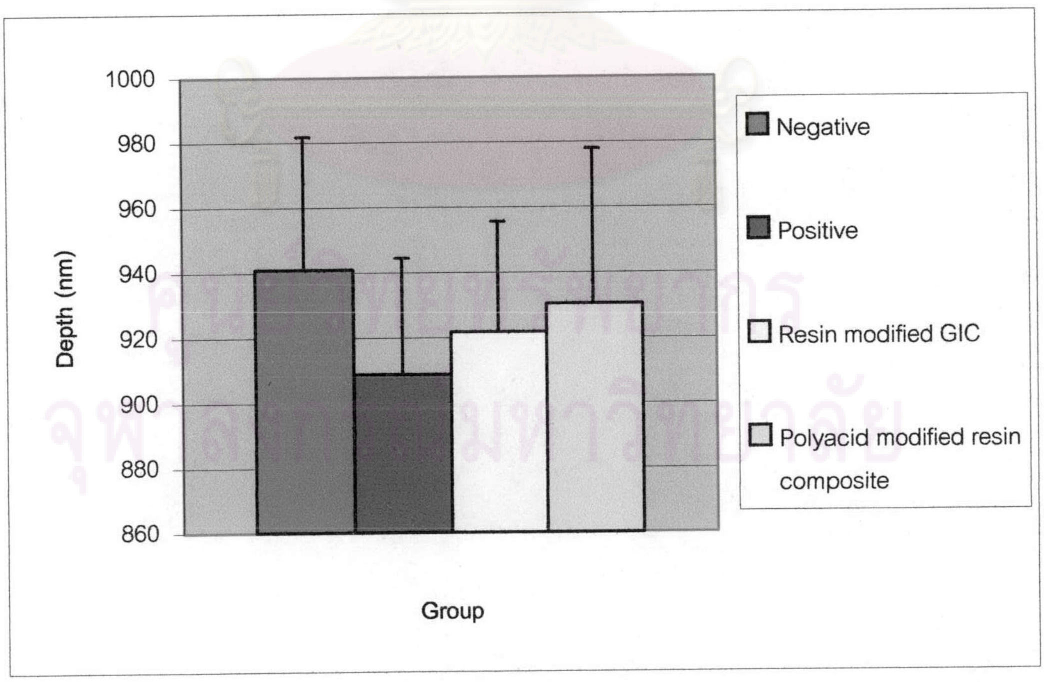

Figure 5.12. Bar graph indicated that there were statistically significant between groups except resin modified glass ionomer cement and polyacid modified resin composite

5.3.1.2. Maximum depth of the series made on enamel by indentation spacing distance of 50 μm

The second attempt to evaluate the surface hardness changes in series next to the restoration in the 6 columns and 4 rows in the area of 300 x 100 microns. The indentation depth of the column and row of each group was showed as a sample in Table 5.6 –5.9. The indentation depth of the negative group was the greatest (911.19 – 1005.27), while the positive was the least (816.32 –874.96). Even though these two groups were the same materials. The resin modified glass ionomer cement and the polyacid modified resin composite group also exhibited the depth in between the negative and positive group.

The statistical comparison was determined by column and row of each group. The results showed that there was not any statistically significant between column or row in each group. However, there was statistically difference between the four groups of enamel in the whole area of the investigation using ANOVA and Bonferroni as the Post Hoc tests (Table 5.10 and Figure 5.13)

Table 5.6. The maximum indentation depth in nanometer (nm) of the enamel specimens for the 6 columns and 4 rows of negative group.

Negative group		Column					
		1	2	3	4	5	6
Row	1	966.50	971.25	981.57	1005.27	993.67	992.53
	2	952.36	944.92	957.73	998.59	987.83	992.28
	3	917.58	920.41	943.67	997.98	975.59	965.87
	4	915.26	911.19	945.68	974.40	957.95	928.53

Table 5.7. The maximum indentation depth in nanometer (nm) of the enamel specimens for the 6 columns and 4 rows of positive group

Positive group		Column					
		1	2	3	4	5	6
Row	1	844.62	851.31	838.84	826.21	869.94	857.83
	2	824.25	853.28	845.86	860.32	874.96	834.29
	3	834.34	846.22	851.29	850.45	842.42	844.82
	4	845.33	816.32	836.65	866.69	852.97	843.51

Table 5.8. The maximum indentation depth in nanometer (nm) of the enamel specimens for the 6 columns and 4 rows of resin modified glass ionomer cement group

Resin modified glass ionomer cement		Column					
		1	2	3	4	5	6
Row	1	839.46	920.39	988.42	946.83	953.38	942.05
	2	852.64	897.02	928.64	902.12	924.73	899.36
	3	879.38	891.54	902.28	882.86	905.68	900.70
	4	864.01	901.32	865.39	867.59	883.55	937.41

Table 5.9. The maximum indentation depth in nanometer (nm) of the enamel specimens for the 6 columns and 4 rows of polyacid modified resin composite

Polyacid modified resin composite		Column					
		1	2	3	4	5	6
Row	1	882.79	983.66	997.90	997.80	979.31	954.96
	2	899.01	1002.74	980.75	991.88	966.71	943.98
	3	883.84	906.31	920.38	912.88	934.73	921.88
	4	874.41	889.31	913.99	895.30	901.75	910.77

Table 5.10. The mean maximum penetration depth of the whole investigation area of all the groups

Group	N	Mean	Standard Deviation	Standard Error	Minimum	Maximum
Negative	119	950.24	62.03	5.69	827.02	1098.79
Positive	120	891.00	36.69	3.35	816.32	985.70
Resin modified glass ionomer cement	143	922.68 *	50.23	4.21	806.92	1184.08
Polyacid modified resin composite	144	932.62 *	55.29	4.61	813.97	1096.54

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

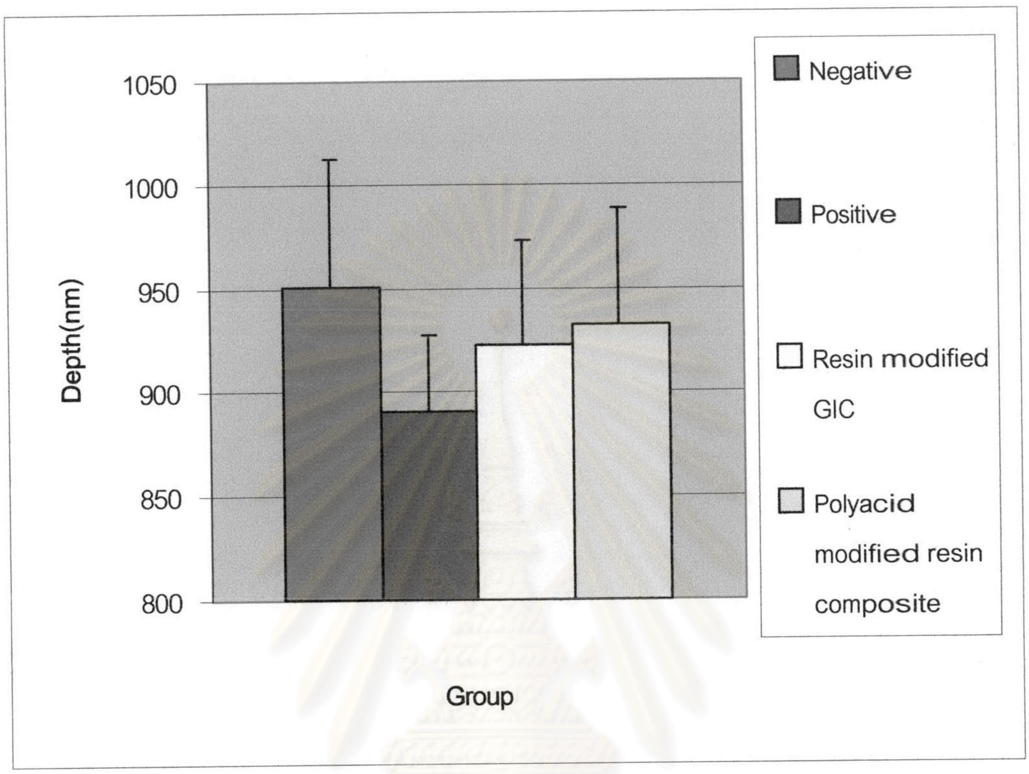

Figure 5.13. Bar graph indicated that there were statistically significant between groups except resin modified glass ionomer cement and polyacid modified resin composite.

For the distance from the enamel-restoration interface, the results showed statistically difference in the resin modified glass ionomer cement and polyacid modified resin composite by means of column. The column next to the restoration (distance of 50 μm) showed significant less penetration depth than the others (Table 5.11 - 5.12 and Figure 5.14). But the negative and positive groups exhibited no statistically difference among all columns (appendix 5.1 and Figure 5.14).

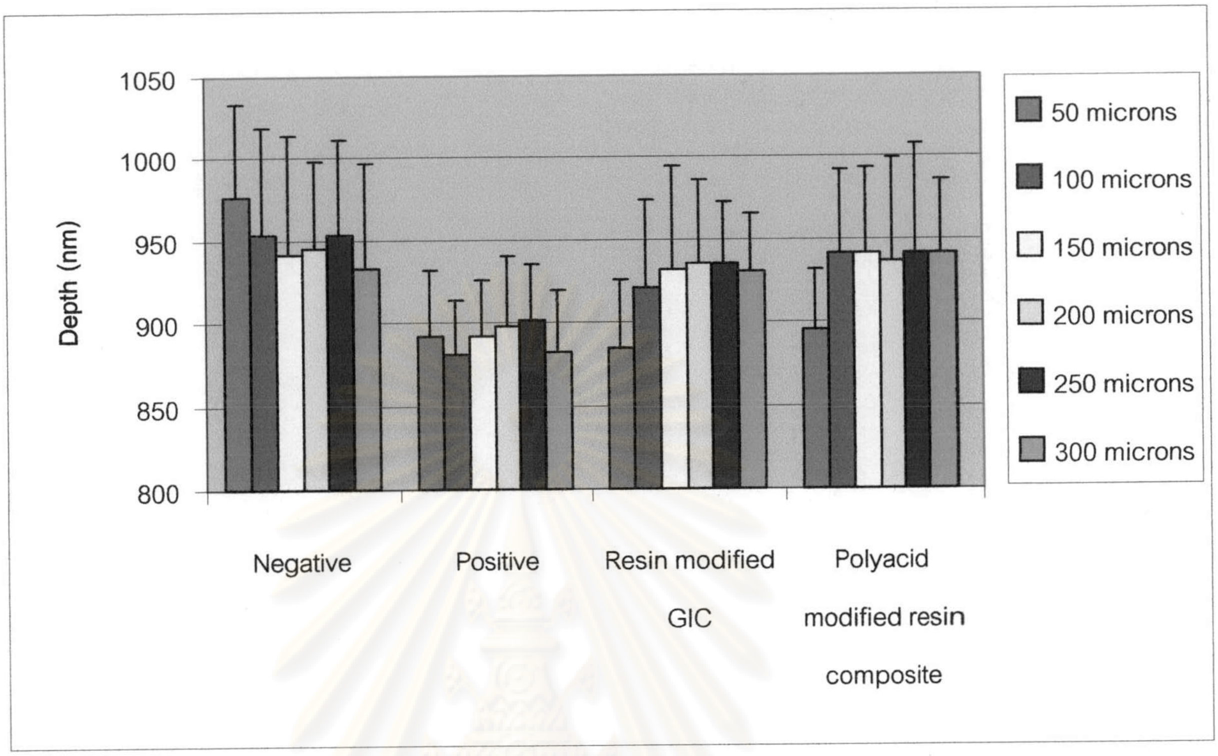

Figure 5.14 Bar graph indicated the subsurface maximum penetration depth as a function of distance from the enamel material interface in 6 columns.

Table 5.11. The statistical comparison between columns at the distance of the restoration from 50 to 300 μm in enamel-resin modified glass ionomer cement interface group

	N	Mean	Standard Deviation	Standard Error	Minimum	Maximum
50 μm	24	884.01 *	41.80	8.53	806.92	981.56
100 μm	24	920.63 * **	53.44	10.91	830.24	1039.2
150 μm	24	931.42 **	62.64	13.06	865.13	1184.08
200 μm	24	935.31 **	50.31	10.27	845.29	1053.07
250 μm	24	934.74 **	37.58	7.67	868.59	992.41
300 μm	24	930.33 **	35.48	7.24	870.02	985.39

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

Table 5.12. The statistical comparison between columns at the distance of the restoration from 50 to 300 μm in enamel-polyacid modified resin composite interface group

	N	Mean	Standard Deviation	Standard Error	Minimum	Maximum
50 μm	24	895.47	35.75	7.30	818.40	979.98
100 μm	24	941.55 *	50.00	10.21	833.97	1002.74
150 μm	24	940.95 *	52.78	10.77	839.26	1026.99
200 μm	24	936.03 *	63.69	13.00	813.97	1004.85
250 μm	24	940.91 *	67.42	13.76	814.56	1096.54
300 μm	24	940.80 *	45.69	9.33	836.76	999.29

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

All the three groups except the positive group exhibited the difference between the penetration depth by mean of row (Table 5.13-5.15 and Figure 5.15). The penetration depth of the row next to the outer surface showed significant more penetration depth than the others.

Table 5.13. The statistical comparison between rows at the distance of the surface 25 to 100 μm in negative group

	N	Mean	Standard Deviation	Standard Error	Minimum	Maximum
25 μm	30	985.78 *	63.83	11.65	850.39	1098.79
50 μm	30	955.82 *,**	66.60	12.16	827.02	1093.01
75 μm	30	932.27 **	50.89	9.29	851.70	1026.04
100 μm	29	926.31 **	48.89	9.08	863.55	1024.55

Note: the same affix showed no significant difference among groups

Table 5.14. The statistical comparison between rows at the distance of the surface 25 to 100 μm in resin modified glass ionomer cement group

	N	Mean	Standard Deviation	Standard Error	Minimum	Maximum
25 μm	35	948.41 *	68.79	11.63	806.92	1184.08
50 μm	36	926.64 *,**	45.70	7.62	810.10	1001.80
75 μm	36	909.91 **	34.83	5.81	843.88	975.36
100 μm	36	906.46 **	34.96	5.83	845.29	969.43

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

Table 5.15. The statistical comparison between rows at the distance of the surface 25 to 100 μm in polyacid modified resin composite group

	N	Mean	Standard Deviation	Standard Error	Minimum	Maximum
25 μm	36	965.34 *	43.39	7.23	879.57	1055.87
50 μm	36	949.63 *	52.41	8.73	855.06	1096.54
75 μm	36	910.02 **	49.81	8.30	821.71	999.03
100 μm	36	905.48 **	51.93	8.65	813.97	988.68

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

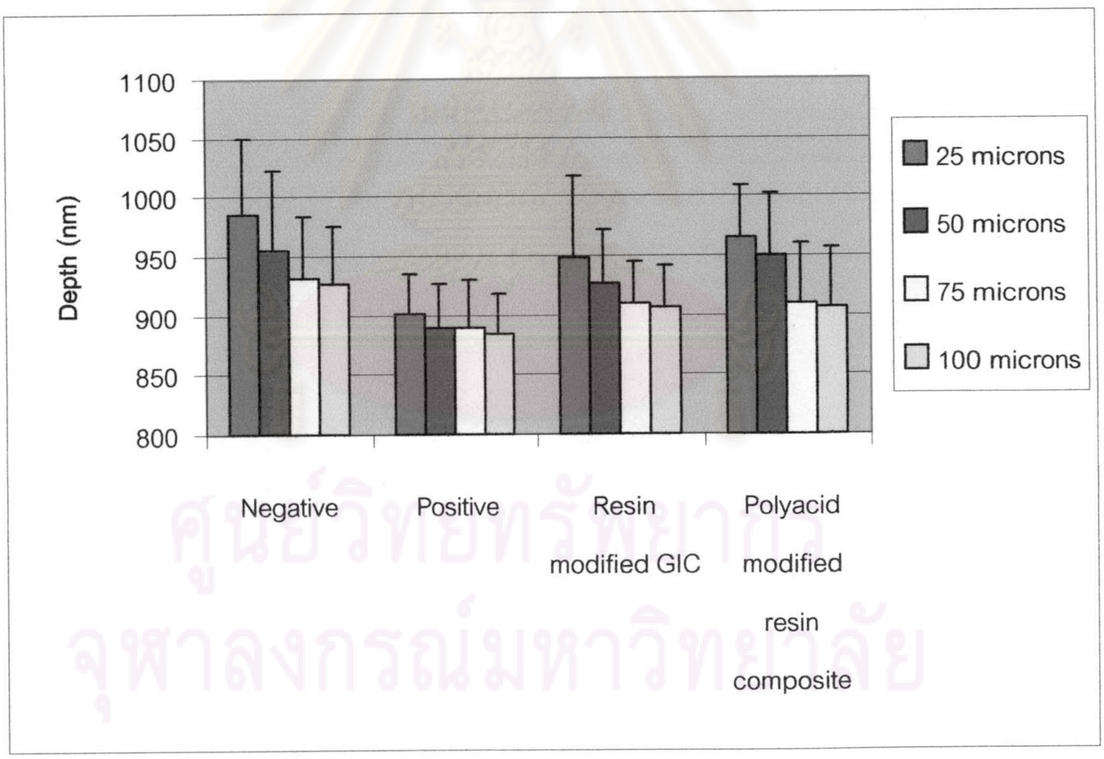

Figure 5.15. Bar graph indicated the subsurface hardness as a function of distance from enamel surface in 4 rows

The first investigation on subsurface enamel (spacing 20 microns), the results of maximum penetration depth were summarized below:

1. For each material, there were no significant difference among the columns (up to 100 microns from the interface) and rows (up to 160 microns from the tooth surface).
2. Determining all the indentations on each material, there was significant difference among the group studied except the mean depth between resin modified glass ionomer cement and polyacid modified resin composite.

The second investigation on subsurface enamel (spacing 50 microns), the results of maximum penetration depth were summarized below:

1. For resin modified glass ionomer cement and polyacid modified resin composite, there were significant difference among the columns (up to 300 microns and rows (up to 100 microns from the tooth surface)
2. The first column (resin modified glass ionomer cement and polyacid modified resin composite) which were within 50 microns from the interface showed less maximum depth compared to other groups (50 – 300 microns from the interface)
3. The first row (all materials except positive group) which were within 25 microns from the tooth surface showed more significantly different depth than other groups (75 – 100 microns from the tooth surface).
4. Determining all the indentations on each material, there was significant difference among the group studied except the mean depth between resin modified glass ionomer cement and polyacid modified resin composite.

5.3.2. Measurement of maximum penetration depth on dentin

5.3.2.1. Maximum depth of the series made on dentin by indentation spacing distance of 15 μm

The maximum depths of each indentations in the column and row of each group were indicated as a sample in Table 5.16 - 5.19. The negative group showed the deepest indentation depth, while the positive group exhibited the least. The indentation depth of the resin modified glass ionomer cement and polyacid modified resin composite groups were almost the same and their depths were between the negative and positive groups (Figure 5.18). However, there were statistically differences among groups of dentin in the whole area of the investigation using ANOVA and Bonferroni as the Post Hoc tests (Table 5.20 and Figure 5.18).

The comparison average of all the average maximum depth for each material in terms of columns and rows were shown in Figure 5.16 - 5.17. The statistical comparison were determined by column and row of each group. The results showed that there was not any statistically significant between columns or rows in each group..

Table 5.16 The maximum indentation depth in nanometer (nm) of the dentin specimens for the 5 columns and 8 rows of negative group

Negative group		Column				
		1	2	3	4	5
Row	1	1276.99	1236.96	1293.74	1301.18	1299.85
	2	1189.87	1196.87	1181.17	1103.63	1170.52
	3	1208.39	1197.99	1227.77	1223.89	1239.25
	4	1176.56	1222.89	1171.61	1193.08	1215.35
	5	1295.38	1262.27	1221.90	1229.96	1188.72
	6	1239.49	1199.46	1193.05	1242.72	1192.18
	7	1286.52	1197.34	1212.75	1140.93	1218.77
	8	1158.54	1156.76	1161.80	1184.16	1169.82

Table 5.17. The maximum indentation depth in nanometer (nm) of the dentin specimens for the 5 columns and 8 rows of positive group

Positive group		Column				
		1	2	3	4	5
Row	1	1037.90	1057.89	1026.27	1015.45	1047.82
	2	1009.23	978.85	1027.32	1033.52	1013.16
	3	1002.98	953.93	995.81	1012.18	978.94
	4	962.13	944.75	1016.97	1011.33	1043.77
	5	1000.65	987.51	996.41	1019.41	994.51
	6	951.77	985.52	1036.36	975.31	973.30
	7	1016.06	996.34	997.75	982.05	1051.89
	8	1034.36	1012.22	1047.34	949.53	941.49

Table 5.18. The maximum indentation depth in nanometer (nm) of the dentin specimens for the 5 columns and 8 rows of resin modified glass ionomer cement group

Resin modified glass ionomer cement		Column				
		1	2	3	4	5
Row	1	1054.87	1034.55	1047.55	1057.44	1066.41
	2	1042.40	1071.88	1086.86	1058.77	1057.78
	3	1035.11	1068.70	1070.71	1045.91	1057.25
	4	1076.27	1070.53	1083.21	1073.74	1054.84
	5	1016.39	1094.72	1087.79	1074.78	1098.61
	6	1080.54	1100.21	1099.90	1057.31	1067.50
	7	1150.63	1060.24	1059.50	1058.45	1076.01
	8	1084.40	1039.85	1002.66	1061.13	1054.99

Table 5.19. The maximum indentation depth in nanometer (nm) of the dentin specimens for the 5 columns and 8 rows of polyacid modified resin composite group

Polyacid modified resin composite	Column					
		1	2	3	4	5
Row	1	1091.94	1116.80	1037.55	1079.42	1101.08
	2	1169.94	1056.84	1053.94	1065.27	1075.45
	3	1043.42	1024.25	1049.91	1083.52	1016.30
	4	1087.54	1086.67	1111.16	1070.60	1036.74
	5	1154.92	1122.11	1132.68	1085.76	1116.12
	6	1149.38	1294.80	1093.48	1066.19	1098.83
	7	1108.67	1138.63	1013.87	1058.61	1132.37
	8	1186.10	1065.99	1040.36	1095.10	1103.62

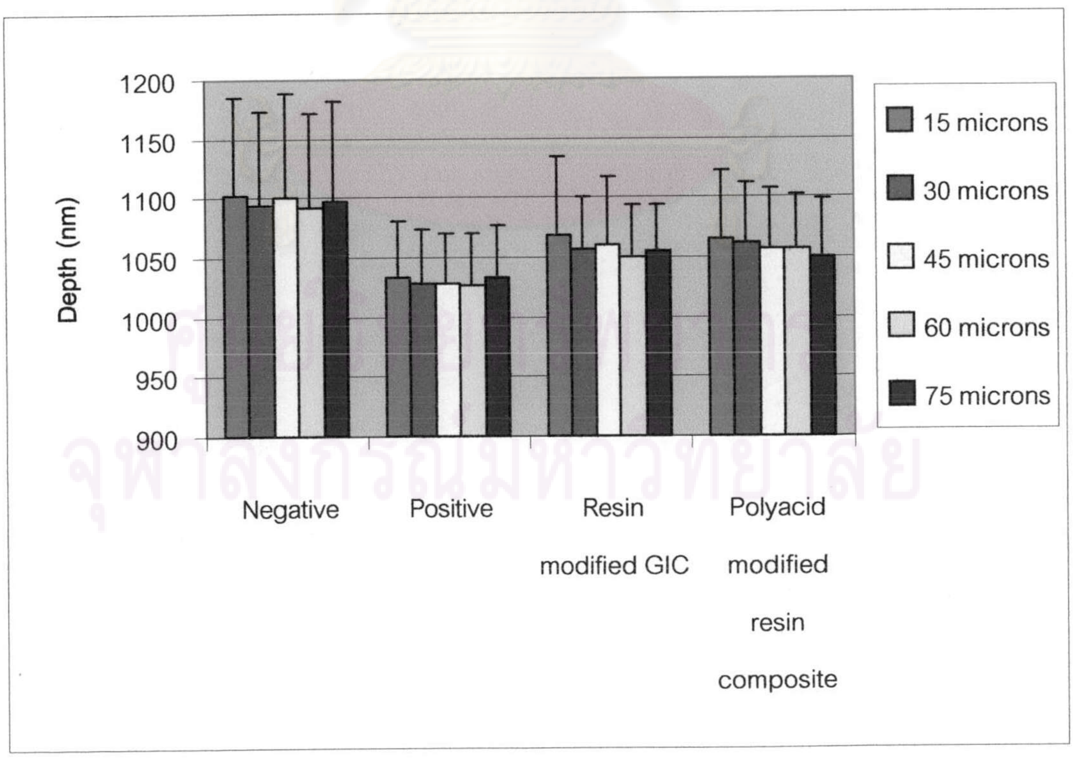

Figure 5.16. Bar graph indicated the subsurface mean penetration depth as a function of distance from the dentin material interface in 5 columns

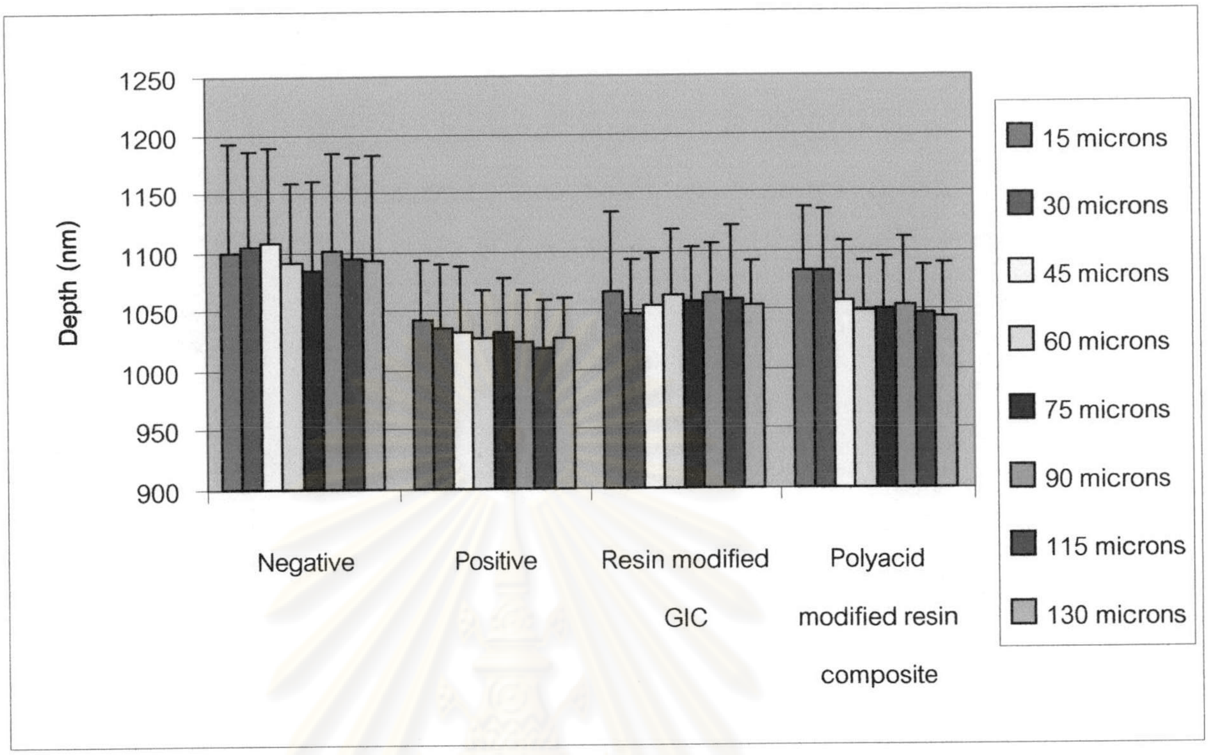

Figure 5.17. Bar graph indicated the subsurface mean penetration depth as a function of distance from the tooth surface in 8 rows

There were no significant differences of penetration depth among the 5 columns and 8 rows of all groups (appendix 5.2). However, there were statistically differences among the four groups of dentin in the investigation area using ANOVA and Bonferroni as shown in Table 5.20 and Figure 5.19.

Table 5.20 The statistical comparison of the indentation depth among the test groups.

Group	N	Mean	Standard Deviation	Standard Error	Minimum	Maximum
Negative	279	1096.96	82.38	4.93	991.02	1301.18
Positive	280	1029.57	44.77	2.68	933.32	1186.37
Resin modified glass ionomer cement	277	1057.97 *	50.92	3.06	924.23	1289.97
Polyacid modified resin composite	280	1058.30 *	50.34	3.01	934.48	1294.80

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

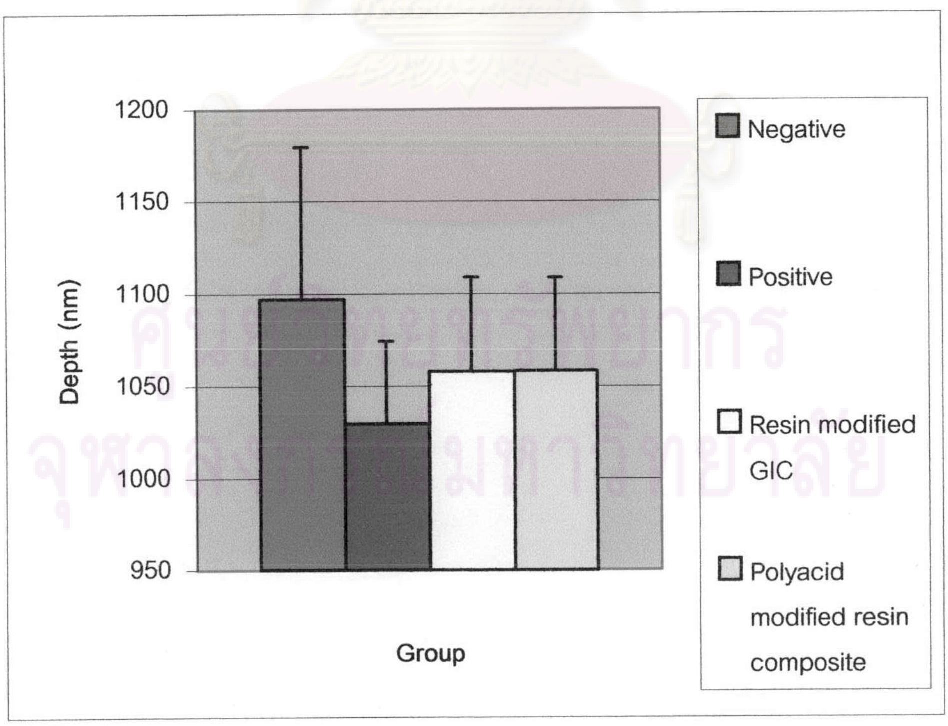

Figure 5.18. Bar graph indicated that there was significant among groups except resin modified glass ionomer cement and polyacid modified resin composite groups

5.3.2.2. Maximum depth of the series made on dentin by indentation spacing distance of 50 μm.

The second attempt to evaluate of the surface hardness changes of dentin specimens in series next to the restoration in the 6 columns and 4 rows in the area of 300 x 100 microns. As the expansion of the investigation, the indentation depth of the column and row of the dentin specimens groups was as the samples (Table 5.21 –5.24). The indentation depth of the negative group was the greatest, while the positive was the least (Figure 5.19). Even though these two groups were the same materials. The resin modified glass ionomer cement and the polyacid modified resin composite groups also exhibited the depth in between the negative and positive group.

Table 5.21. The maximum indentation depth in nanometer (nm) for the 6 columns and 4 rows of dentin negative specimens

Negative group		Column					
		1	2	3	4	5	6
Row	1	1129.30	1206.90	1157.37	1147.96	1166.42	1205.88
	2	1027.73	1194.86	1151.53	1140.20	1140.29	1186.84
	3	1017.07	1207.70	1154.10	1141.52	1135.40	1143.88
	4	1106.79	1214.00	1182.57	1145.47	1103.06	1118.07

Table 5.22. The maximum indentation depth in nanometer (nm) for the 6 columns and 4 rows of dentin positive specimens.

Positive group		Column 1	2	3	4	5	6
Row	1	879.70	872.97	846.54	889.37	811.79	822.29
	2	854.22	866.05	849.96	907.07	879.05	857.21
	3	858.43	849.53	872.69	877.96	856.82	897.05
	4	896.51	844.3	876.17	810.97	879.23	838.34

Table 5.23. The maximum indentation depth in nanometer (nm) for the 6 columns and 4 rows of dentin resin modified glass ionomer cement specimens

Resin modified glass ionomer cement		Column 1	2	3	4	5	6
Row	1	860.67	838.97	939.98	930.51	933.99	959.91
	2	880.34	858.90	892.82	950.04	908.58	908.15
	3	880.04	901.28	893.93	885.91	931.77	873.21
	4	860.82	890.81	964.34	909.67	910.13	879.78

Table 5.24. The maximum indentation depth in nanometer (nm) for the 6 columns and 4 rows of dentin polyacid mofied resin composite specimens.

Polyacid mofied resin composite		Column					
		1	2	3	4	5	6
Row	1	835.23	855.84	927.79	919.52	963.86	930.30
	2	837.15	852.88	971.54	897.24	922.63	934.76
	3	879.43	842.51	906.89	894.85	902.69	966.46
	4	880.24	856.42	918.33	930.30	894.30	930.20

Table 5.25. The mean maximum penetration depth of the whole area of dentin specimens groups.

	N	Mean	Std Deviation	Minimum	Maximum
Negative	119	1078.51	87.57	981.78	1245.64
Positive	120	883.92	50.75	761.08	1001.91
Resin modified glass ionomer cement	168	920.60 *	59.81	820.61	1086.20
Polyacid modified resin composite	168	929.26 *	59.08	802.26	1096.86

Note : The same affix showed no significant difference.

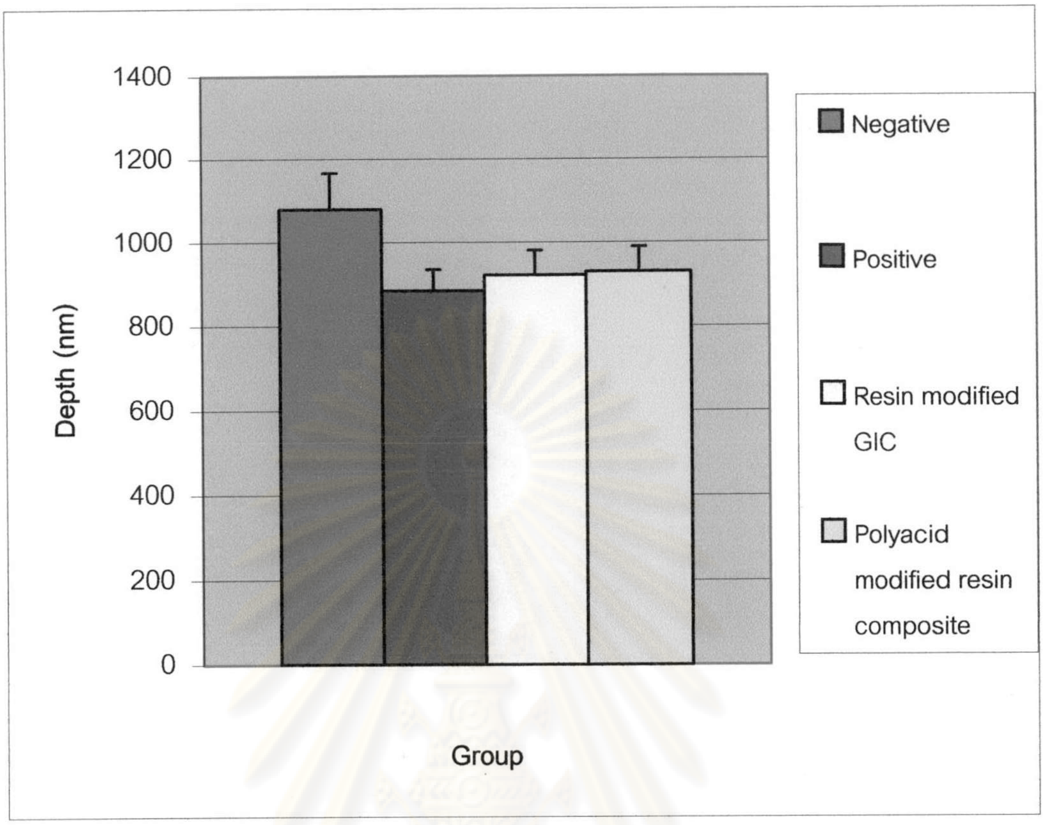

Figure 5.19. Bar graph indicated that there were statistically significant between groups except resin modified glass ionomer cement and polyacid modified resin composite

For the distance from the dentin-restoration interface, the results showed statistically difference in the resin modified glass ionomer cement and polyacid modified resin composite by means of column. The column next to the restoration (distance of 50 μm) showed significant less penetration depth than the others (Table 5.26 - 5.27 and Figure 5.20). But the negative and positive groups exhibited no statistically difference among all columns (appendix 5.4 and Figure 5.20).

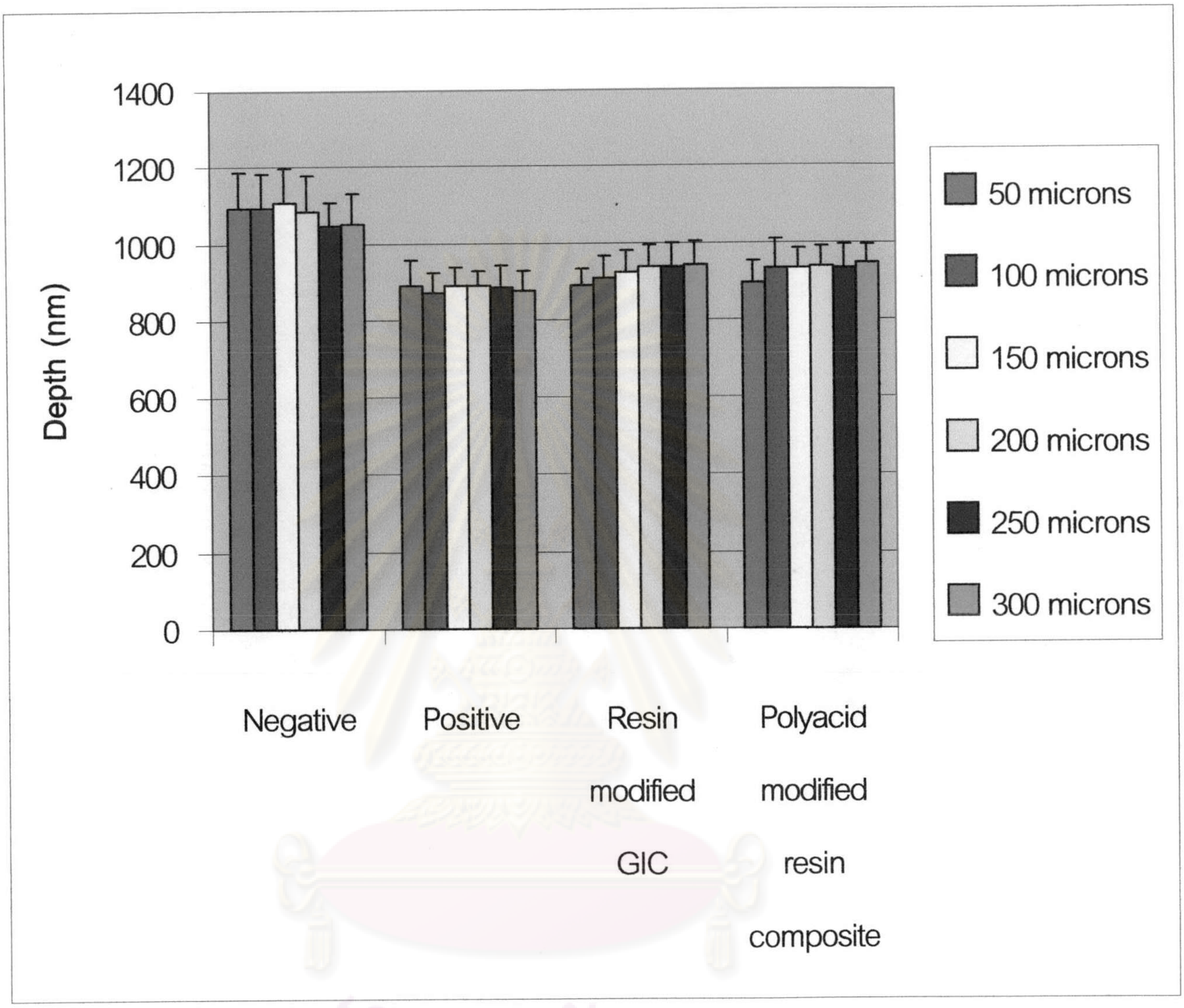

Figure 5.20 Bar graph indicated the subsurface maximum penetration depth as a function of distance from the dentin material interface in 6 columns

Table 5.26. The statistical comparison between column at the distance of the restoration from 50 to 300 μm in dentin-resin modified glass ionomer cement interface group

	N	Mean	Standard Deviation	Standard Error	Minimum	Maximum
50 microns	28	886.84 *	44.02	8.32	826.30	979.27
100 microns	28	906.94 *,**	58.67	11.09	820.69	1024.45
150 microns	28	922.08 *,**	56.53	10.68	851.06	1072.81
200 microns	28	934.19 **	57.29	10.83	868.17	1086.20
250 microns	28	934.28 **	64.14	12.12	822.74	1059.82
300 microns	28	939.23 **	63.47	11.99	821.69	1041.90

Note : The same affix showed no significant difference.

Table 5.27. The statistical comparison between column at the distance of the restoration from 50 to 300 μm in dentin-polyacid modified resin composite interface group

	N	Mean	Standard Deviation	Standard Error	Minimum	Maximum
50 microns	28	896.13 *	55.82	10.55	802.26	997.45
100 microns	28	932.00 *	75.49	14.27	808.38	1096.86
150 microns	28	930.74 *	54.06	10.22	804.54	1027.02
200 microns	28	937.80 *	49.44	9.34	855.26	1032.88
250 microns	28	931.72 *	60.96	11.52	838.03	1089.07
300 microns	28	947.16	46.13	8.72	862.35	1044.49
300 microns	28	947.16	46.13	8.72	862.35	1044.49

Note : The same affix showed no significant difference.

All the three groups except positive group also showed the difference between the depth of the effect of demineralization solution in 50 microns to 200 microns (Table 5.28-

5.30 and Figure 5.21). The row indentation depth of positive group was found that there was not any statistically significant different as in appendix 5.4.

Table 5.28. The statistical comparison between rows at the distance of the surface 25 to 100 microns in negative group

	N	Mean	Standard Deviation	Standard Error	Minimum	Maximum
25 microns	29	1128.58 *	91.38	16.97	1001.15	1245.64
50 microns	30	1102.37 *,**	87.67	16.01	993.65	1242.73
75 microns	30	1049.91 **,***	72.33	13.21	981.78	1207.70
100 microns	30	1034.85 ***	65.65	11.99	986.25	1214.00

Note: The same affix showed there were no statistically significant difference.

Table 5.29. The statistical comparison between rows at the distance of the surface 25 to 100 micron in resin modified glass ionomer cement group

	N	Mean	Standard Deviation	Standard Error	Minimum	Maximum
25 microns	42	945.35 *	65.65	10.13	821.69	1072.81
50 microns	42	925.39 *, **	59.26	9.14	837.54	1086.20
75 microns	42	905.40 **	54.72	8.44	820.69	1032.42
100 microns	42	906.23 **	51.52	7.95	838.10	1025.78
100 microns	42	906.23 **	51.52	7.95	838.10	1025.78

Note: The same affix showed there were no statistically significant difference.

Table 5.30. The statistical comparison between rows at the distance of the surface 25 to 100 micron in polyacid modified resin composite group

	N	Mean	Standard Deviation	Standard Error	Minimum	Maximum
25 microns	42	951.55 *	71.30	11.00	804.54	1096.86
50 microns	42	938.62 *, **	55.99	8.64	831.82	1089.07
75 microns	42	915.08 **	50.38	7.77	808.38	1024.09
100 microns	42	911.78 **	48.39	7.47	802.26	996.64

Note: The same affix showed there were no statistically significant difference.

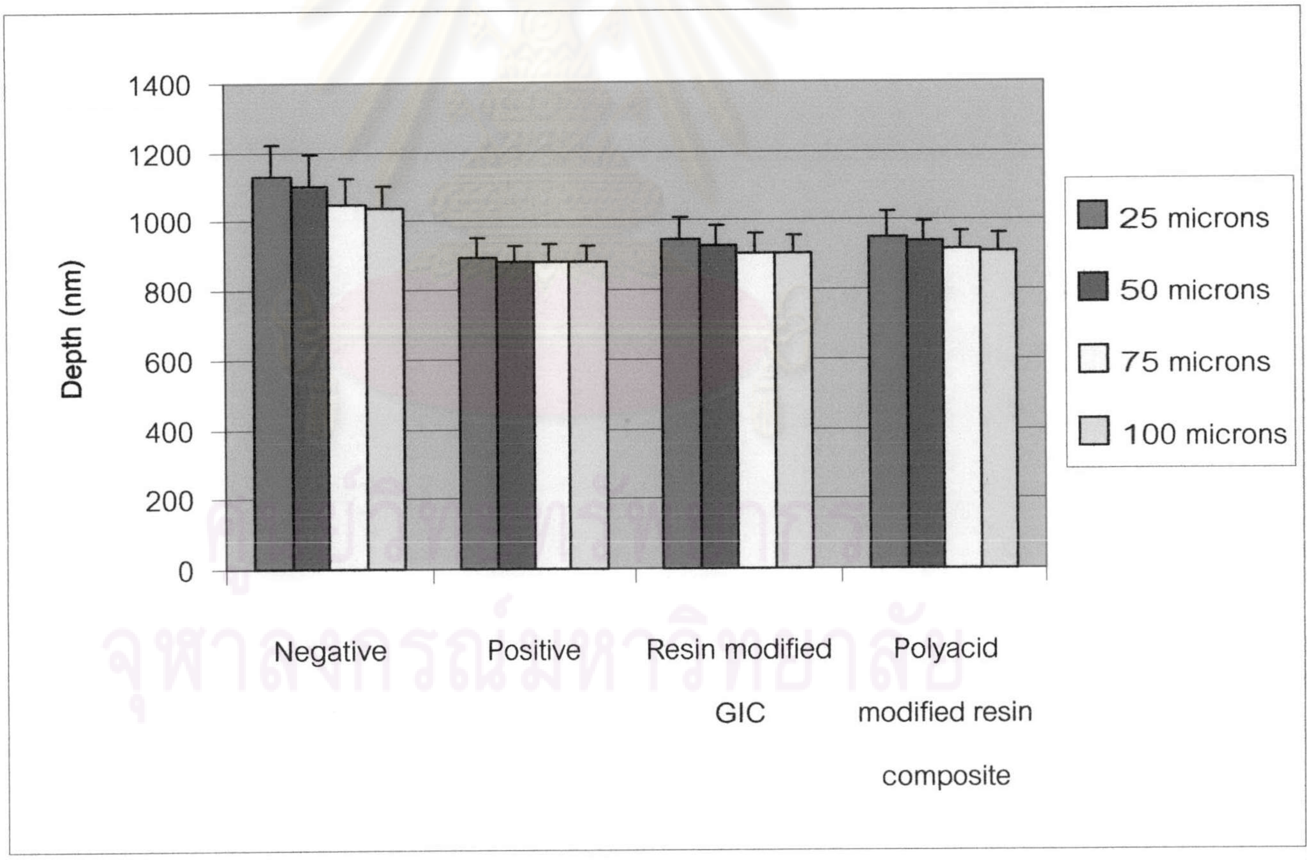

Figure 5.21. Bar graph indicated the subsurface maximum penetration depth as a function of distance from the restoration of 4 rows

The first investigation on subsurface dentin, the results of maximum penetration depth were summarized below:

1. For each material, there were no significant difference among the columns (up to 75 microns from the interface) and rows (up to 130 microns from the tooth surface).
2. Determining all the indentations on each material, there was significant difference among the group studied except the mean depth between resin modified glass ionomer cement and polyacid modified resin composite.

The second investigation on subsurface dentin, the results of maximum penetration depth were summarized below:

1. For resin modified glass ionomer cement and polyacid modified resin composite, there were significant difference among the columns (up to 300 microns and rows (up to 100 microns from the tooth surface)
2. The first column (resin modified glass ionomer cement and polyacid modified resin composite) which were within 50 microns from the interface showed less maximum depth compared to other groups (50 – 300 microns from the interface)
3. The first row (all materials except positive group) within 25 microns from the tooth surface showed more significantly different depth than other groups (75 – 100 microns from the tooth surface).
4. Determining all the indentations on each material, there was significant difference among the group studied except the mean depth between resin modified glass ionomer cement and polyacid modified resin composite.

5.4. Discussion

In the previous chapter, enamel and dentin next to the fluoride releasing materials were significantly harder within the distance of 100 μm after soaking in the demineralization solution. Therefore there may be some effect on enamel and dentin underneath. In this chapter, the method was designed to investigate the hardness changes of subsurface enamel and dentin. The sample was cross sectional cut through the restoration in order to obtain the subsurface tooth structure underneath. One side of the restoration was left untouched in the previous chapter. Therefore the subsurface tooth structure was not pressured by microindentation hardness test and thus the subsurface hardness can be investigated without pressure or impression on the surface.

Since there were significant changes of hardness at the surface, there was also expectation to see some changes in the subsurface area. Unlike the surface hardness changes of tooth structure after soaking in the demineralization solution, the subsurface of tooth structure was expected to see less change due to the difficulty in transportation of fluoride ion. As a result, nanohardness test was applied to investigate the small area next to the interface since the nanohardness test can perform smaller size of indentation. The small indentation spacing can be obtained as small as 20 μm while the microhardness cannot. With this technology, the effect of fluoride releasing materials on three dimension of the adjacent tooth structure can be investigated.

Considerable research such as Arends, Schuthof and Jongebloed (1980b); Forsten (1995); ten Cate and van Duinen (1995) have investigated the demineralization and remineralization of enamel and dentin. However, the microhardness of the inhibition zones influenced by the fluoride releasing materials in the three dimensions of the human enamel and dentin has not been reported. Arends, Schuthof and Jongebloed, (1980b) reported that there was relationship between the lesion depth of artificial enamel caries and Knoop microhardness. The difference was the Knoop impression size was still larger than the Berkovich impression. Measuring the small impression using eye measurement in microhardness technique may give more bias to the result. Therefore, measuring the small size of indentation impression, nanohardness test has an advantage over microhardness test because it uses depth sensing method by linear variable differential transformer (LVDT) which will provide the depth of penetration with precise measurement and less bias. The depth created on the surface can tell the hardness. The more depth created on the surface, the less resistance belongs to the material. The outcome of the material is softer. In this chapter, the depth was therefore considered to be the important factor to consider the resistance to pressure. Another reason was nanohardness test provided different type of hardness value.

In the first investigation, the results exhibited that there were no statistically differences of the subsurface penetration depth in the individual group of 100 μm next to the restoration in both enamel and dentin. The first impression was coming to the conclusion that there was no changes in the internal surface within 100 μm from the material tooth interface. The second attempt of the investigation followed the same method but the area was extended to 300 μm in order to see the difference. The results exhibited that resin modified glass ionomer cement group showed some effect on the penetration depth at the column of 50 μm compared to those of 150, 200, 250 and 300 μm. However, there were no statistically difference in 50 and 100 μm next to the restoration which confirmed the result of the first investigation. The results of resin modified glass ionomer cement produced an inhibition zone with a greater microhardness with those formed adjacent to the polyacid modified resin composite. The result was correlated to the result of Pereira et al. in 1998. They studied in bovine dentin with the conventional glass ionomer cement and resin modified glass ionomer cement with the Knoop tester parallel to the cavity wall. They found that the level of inhibition zone was in the area of 300 μm and the effective of the resistance was material independent. Many investigators reported of caries resistance in vitro of fluoride releasing material by polarized light microscope (Hicks, 1986a; Marinelli et al., 1997; Millar, Abiden and Nicholson, 1998; Pereira et al., 1998; Tam, Chan and Yim, 1997). Their results concluded that the fluoride releasing materials imparted resistance against the development of carious lesion in vitro.

Previous reports have suggested that glass ionomer cement may promote remineralization by possibly depositing minerals and fluoride, therefore increasing the acid resistance (ten Cate and van Duinen, 1995, Modesto et al, 1997). Diaz-Arnold et al., (1995a) observed that a conventional glass ionomer cement released greater amount of fluoride than resin modified glass ionomer cement. However, Forsten (1995) found that the fluoride levels released by resin modified glass ionomer cement were higher or the same as the conventional glass ionomer cement. Therefore, the explanation of their result in fluoride released may be the different fluoride content of the materials and the different methods used to determine fluoride release. In this study, the surface hardness was used to determine the effect of fluoride releasing materials instead of direct method of fluoride released and uptake. The results also showed that the subsurface hardness of enamel and dentin next to the fluoride releasing materials were harder as well as their surface hardness. And we also found that the affected surface was in the limited area. The increase of hardness of the surface enamel and dentin next to restoration is expected to the result from the fluoride. Fluoride bound to minerals could promote less solubility in enamel and dentin. Fluoroapatite as a result of remineralization process is also harder and more acid resistance. The effect of fluoride on hardness is therefore needed further investigation.