

## Effect of glass ionomer cements on nanohardness of caries-affected dentin

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**Purpose:** To evaluate nanohardness of caries-affected dentin adjacent to conventional and resin-modified glass ionomer cements.

**Materials and Methods:** Dentin caries was removed from the occlusal surfaces of human molars. Teeth were cut mesio-distally through the middle of prepared cavities to get two halves. One half was filled with the tested material, either Fuji IX GP or Fuji II LC, while the other half was unfilled and used as a negative control (n=7). After 1, 7 and 30 days of restoration, nanohardness was measured on intertubular dentin at 20, 40, 60, 80 and 100 µm from the pulpal margin. Mean nanohardness at each distance of each period were compared using analysis of variance (ANOVA) and Fisher's least significant difference (LSD) multiple comparison tests at significant level of 5%.

**Results:** Nanohardness of caries-affected dentin adjacent to both materials increased after restoration for seven days. Significant increases were observed after 30 days of restoration. Nanohardness change of caries-affected dentin adjacent to Fuji IX GP was more than that adjacent to Fuji II LC.

**Conclusion:** The nanohardness of caries-affected dentin adjacent to conventional and resin-modified glass ionomer cements increased after seven days of restoration. (Int Chin J Dent 2003; 3: 122-130.)

**Clinical Significance:** Application of glass ionomer cements on caries-affected dentin can increase hardness of such dentin.

**Key Words:** caries-affected dentin, glass ionomer cement, nanohardness.

### Introduction

Glass ionomer cements are frequently used as a restorative material, especially in highly caries-risk patients.<sup>1</sup> Chemical bonding to tooth structures is one of their advantageous properties.<sup>2-5</sup> Cariostatic effect and remineralization promotion due to gradual release of fluoride are their other favorable properties.<sup>6-8</sup> Several studies have reported demineralization inhibition of normal tooth structures<sup>9,10</sup> and remineralization of enamel lesions adjacent to glass ionomer cements.<sup>11,12</sup>

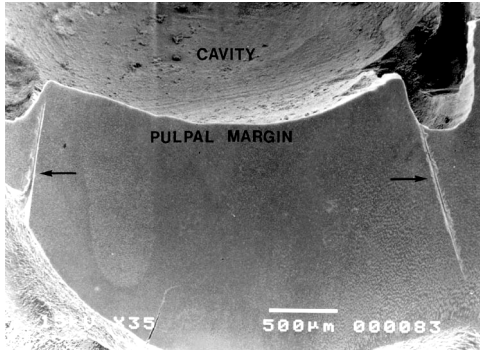
Clinically, carious dentin is a common substrate to be dealt with when treating the patients. It is classically described as consisting of two layers: the outer carious dentin or infected dentin and the inner carious dentin or affected dentin.<sup>13</sup> The outer carious dentin, which is irreversibly denatured, unremineralisable, infected, insensitive, and necrotic; should be completely removed.<sup>14,15</sup> The inner carious dentin, which is reversibly denatured, remineralisable, uninfected, sensitive, and vital; should be preserved.<sup>16</sup> Using a caries detector, which stains infected dentin, enables us to remove the outer carious dentin while preserving the caries-affected dentin.<sup>17</sup> Caries-affected dentin has a lower hardness value than normal dentin since it is partially demineralized.<sup>18-20</sup>

*In vitro* studies using artificial dentin caries models have demonstrated remineralization and hyper-mineralization of dentinal lesions adjacent to glass ionomer cement restorations.<sup>21,22</sup> *In vivo* studies, physiological remineralization of caries-affected and artificial demineralized dentin, after restoring using several kinds of materials, have also been reported.<sup>14,16,23-25</sup> These studies were mostly done in dogs and monkeys, and only one study has been performed on human teeth.<sup>16</sup> The results showed that mineral content recovering of the lesions resulted in an increase of hardness. This type of remineralization was stated to be the result of either normal biological processes or the process that was initiated by mineral released from restorative materials.<sup>14</sup> However, the effect of glass ionomer restoration on hardness or remineralization of caries-affected dentin of human teeth has not been clarified. Therefore, the purpose of this study was to investigate the nanohardness of caries-affected dentin of human teeth adjacent to glass ionomer restorative cements.

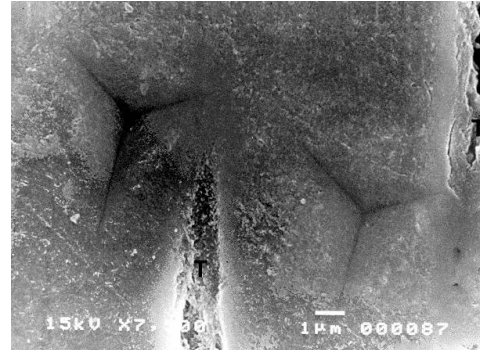
## Materials and Methods

### Specimen Preparation

Fourteen extracted human molars having occlusal carious lesions involving middle dentin, which were kept frozen less than one month, were randomly divided into two groups. Caries was removed with slow speed steel round burs (Meisinger ISO #016, 014, 012, and 010, Hager & Meisinger GmbH, Dusseldorf, Germany) using caries detector solution (Caries Detector, Kuraray Medical, Tokyo, Japan) as an indicator. Each tooth was mesio-distally sectioned through the middle of the prepared cavity into two halves using a low speed diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA). The cavities on one half of the teeth in the first group were filled with Fuji IX GP (GC Corp., Tokyo, Japan) and those in the second group were filled with Fuji II LC (GC Corp.) according to the manufacturer's recommendations. The cavities on the other half of the teeth in both groups were unfilled and used as a negative control. Each specimen was embedded in epoxy resin (Epon 815, Nisshin EM, Tokyo, Japan). After 24 hours, the surface of the specimen was polished with consecutive 600, 800, 1,000, and 1,200 grit waterproof silicon-carbide papers (Struers A/S, Copenhagen, Denmark) under tap water, followed by diamond paste particle sizes 6, 3, and 1  $\mu\text{m}$  (DP- Paste, P, Struers A/S). Marks were made by a razor blade under a light microscope (x20) at both ends of the pulpal cavity wall (Fig. 1). Each specimen was kept separately in deionized water at 37°C throughout the 30-day experimental period. Storage water was changed weekly to refresh the environment.



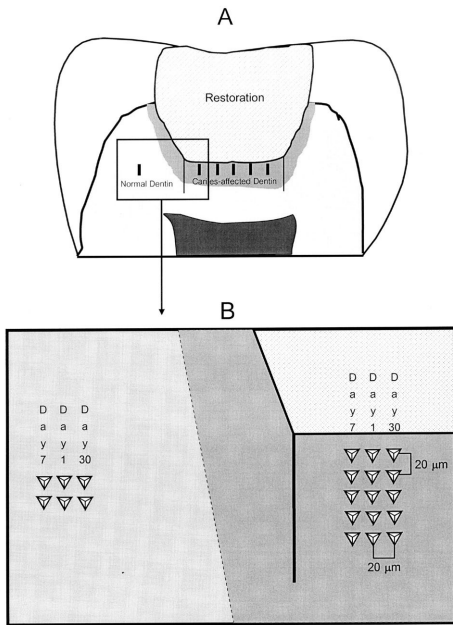
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**Fig. 1.** Marks (arrows) were made at both ends of pulpal margin as a reference for nanohardness indentation on caries-affected dentin (x35).

**Fig. 3.** Indentation marks on intertubular dentin, T = dentinal tubule. (x7,500).



**Fig. 2.**

A. Nanohardness measurements were made at six specific areas. Five areas were on caries-affected dentin and one area was on normal dentin.

B. Each measurement area consists of three columns of indentation marks. Each column represents the indentation at each period of testing (1, 7 and 30 days). Five indentations per column were performed on caries-affected dentin and two indentations per column were performed on normal dentin. Distance between each indentation was 20 μm.

**Hardness Measurements**

Nanohardness of caries-affected and normal dentin of all specimens was measured at 1, 7 and 30 days after restoration using a nanoindentation tester (ENT-1100, Elionix Corp., Tokyo, Japan). Fig. 2 illustrates simulated indentation areas of restored specimens. Fig. 3 shows an SEM picture of the indentation marks on intertubular dentin (x7,500). At day 1, the indentation positions were set on intertubular dentin of caries-affected dentin at distances of 20, 40, 60, 80 and 100 μm from the pulpal cavity margin. Five indentations at each distance, a total of 25 indentations, were performed between the marks. For normal-dentin hardness measurement, the indentation positions were set on the intertubular dentin beside caries-affected dentin, and two indentations were performed. Positions of indentation at day 7 and day 30 were set along side those of day 1. Indentations in the control specimens were done using the same procedure as that of the restored specimen. After indentation performing, nanohardness values were

calculated.

**Statistical Analysis**

Mean nanohardness at each distance and each period was calculated. The data were compared by one-way analysis of variance (ANOVA) and Fisher's least significant difference (LSD) multiple comparison tests (p=0.05). Statistical tests were performed using a computerized statistical program (SPSS for Windows release 8.0.0, SPSS Inc., USA).

**Results**

Mean nanohardness of caries-affected and normal dentin of the Fuji IX GP and Fuji II LC groups are shown in Tables 1 and 2, respectively. Nanohardness of caries-affected dentin increased when measured from cavity wall toward deep dentin. During the 30 days of this study, there was no significant change of nanohardness in the control specimens of both groups. Increases in nanohardness of caries-affected dentin of the restored specimens in both groups were observed after 7 and 30 days. Fig. 4 shows the nanohardness of caries-affected dentin adjacent to Fuji IX GP at 1, 7 and 30 days. After restoration for seven days, nanohardness at all measured distances increased but were not significantly different from those of day 1 (p>0.05).

**Table 1.** Mean nanohardness of caries-affected and normal dentin of Fuji IX GP group.

Distance (µm)	Controlled Day 1	Restored Day 1	Controlled Day 7	Restored Day 7	Controlled Day 30	Restored Day 30
20	30.27 (4.69)	30.38 (4.47) <sup>a</sup>	30.10 (4.91)	34.61 (4.97)	30.66 (4.77)	37.42 (3.88) <sup>a</sup>
40	31.95 (5.12)	31.69 (4.85) <sup>b</sup>	31.58 (5.22)	35.43 (5.48)	32.63 (4.95)	37.99 (4.82) <sup>b</sup>
60	33.01 (4.35)	32.92 (4.68) <sup>c</sup>	33.37 (4.79)	35.89 (4.52)	33.58 (5.16)	39.69 (4.89) <sup>c</sup>
80	34.88 (4.19)	34.73 (4.29) <sup>d</sup>	34.05 (4.48)	36.14 (4.93)	34.35 (5.29)	39.22 (3.54) <sup>d</sup>
100	34.60 (4.37)	34.84 (4.17) <sup>e</sup>	34.50 (4.46)	37.22 (4.73)	34.52 (5.06)	39.70 (4.40) <sup>e</sup>
Normal Dentin	59.89 (5.07)	59.36 (5.24)	59.93 (5.51)	59.33 (5.06)	60.23 (5.72)	60.05 (5.44)

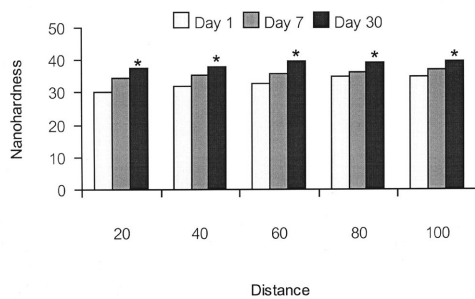
Data having the same letter are significantly different (p<0.05).

**Table 2.** Mean nanohardness of caries-affected and normal dentin of Fuji II LC group.

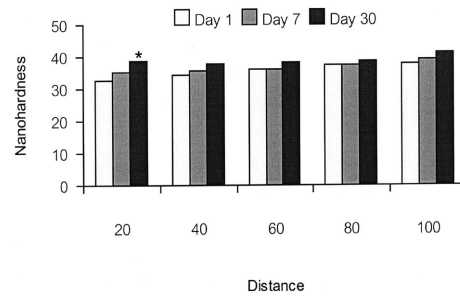
Distance (µm)	Controlled Day 1	Restored Day 1	Controlled Day 7	Restored Day 7	Controlled Day 30	Restored Day 30
20	32.08 (4.11)	32.66 (4.04) <sup>a</sup>	32.34 (4.17)	35.41 (6.78)	32.75 (4.19)	38.69 (4.76) <sup>a</sup>
40	34.57 (4.06)	34.22 (4.71)	34.90 (3.16)	35.76 (5.32)	34.76 (4.16)	37.62 (2.84)
60	35.67 (4.49)	35.89 (3.98)	36.28 (3.19)	35.97 (5.40)	36.48 (4.83)	38.20 (2.87)
80	37.35 (4.26)	37.32 (4.95)	37.29 (3.06)	37.25 (5.11)	37.77 (4.45)	38.74 (3.33)
100	38.93 (3.95)	39.02 (5.03)	38.78 (3.81)	39.22 (5.29)	39.29 (6.08)	41.45 (3.10)
Normal Dentin	61.06 (5.07)	61.46 (5.51)	61.87 (5.59)	61.30 (5.27)	61.58 (5.46)	61.18 (5.42)

Data having the same letter are significantly different (p<0.05).

After restoration for 30 days, nanohardness values at every measured distance were significantly higher than those of day 1 ( $p < 0.05$ ), but not significantly different from those of day 7 ( $p > 0.05$ ). Fig. 5 shows the results of nanohardness of caries-affected dentin adjacent to Fuji II LC. Nanohardness at all measured distances increased after restoration for seven days but were not significantly different from those of day 1 ( $p > 0.05$ ). After restoration for 30 days, nanohardness of only the 20  $\mu\text{m}$  distance was significantly higher than that of day 1 ( $p < 0.05$ ) but was not significantly different from that of day 7 ( $p > 0.05$ ). After 30 days, nanohardness of caries-affected dentin adjacent to restorations was lower than that of normal dentin.



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**Fig. 4.** Nanohardness of caries-affected dentin adjacent to Fuji IX GP.

**Fig. 5.** Nanohardness of caries-affected dentin adjacent to Fuji II LC.

\* : Significantly different ( $p < 0.05$ ) for comparison between nanohardness at day 30 and day 1 for both figures.

### Discussion

To investigate demineralization and remineralization of teeth *in vitro* and *in vivo* studies, several methods have been used including hardness test,<sup>10</sup> microradiography,<sup>26</sup> contact microradiography,<sup>22</sup> energy dispersive spectroscopy (EDS),<sup>24</sup> and electron-probe microanalysis (EMPA).<sup>25</sup> Hardness of enamel and dentin is associated with mineral change. Demineralization decreases hardness while remineralization increases hardness.<sup>10,24,25,27</sup> Our study used the nanohardness test for evaluation of nanohardness of caries-affected dentin. The nanoindentation tester allows measurements in small areas such as the dentin-enamel junction, inter- and intra-tubular dentin with ultra light loads,<sup>28,29</sup> and provided useful data that could not be obtained using the classical type of hardness tester.<sup>30</sup> Measured areas in this study was focused and set on intertubular dentin (Fig. 3). Recent works<sup>27,31,32</sup> indicated that dentin properties are largely dependent on the properties of intertubular dentin. Mineralization appears to increase substantially in caries-affected dentin, and such an increase can be accounted for only if intertubular dentin becomes hypermineralized. The result of this study showed that nanohardness of caries-affected dentin was about half that of normal dentin. This finding is in agreement with other previous studies and clarified the substrate that we wanted to study.<sup>20,33</sup> This substrate was partially demineralized and can be remineralized under suitable conditions. The hardness of the studied area in all groups increased when measured toward deep dentin. This data indicated an approaching to normal dentin that lies under caries-affected dentin.<sup>18</sup>

Nanohardness of the control specimens in both groups did not show any significant change during the period of this study ( $p>0.05$ ). This demonstrates non-effectiveness on remineralization of the storage solution (deionized water), which was used in this study. Nanohardness of caries-affected dentin adjacent to the tested materials tended to increase after seven days of restoration, however, no significant increasing was shown ( $p>0.05$ ). After 30 days of restoration, a significant increase of nanohardness was observed at every measured distance for Fuji IX GP group and at 20  $\mu\text{m}$  from pulpal margin for Fuji II LC group ( $p<0.05$ ). The increase of nanohardness showed signs of remineralization, which needed a period of time to occur. The increasing nanohardness or remineralization that took place in the restored specimens seems to be due to the effectiveness of the filling materials, when compare to the hardness of the controlled specimens in the same environment. Several *in vivo* studies demonstrated physiological remineralization of artificial and normal demineralized tooth structures.<sup>14,23-25</sup> These authors proposed that in the closed environment, minerals (mainly calcium) were delivered from pulp tissue under odontoblastic-process initiation. The data of their studies showed that full re-calcification of the lesion *in vivo* could take place within several months. In our study, without a physiological environment, remineralization of caries-affected dentin adjacent to filling materials could take place in a short period of time. This may be due to the effect of the tested glass ionomer materials that may provide some mineral ions, which become involved in the remineralization of caries-affected dentin. Several mineral ions are released from glass ionomer cements including fluoride, sodium, silicon, calcium strontium, aluminum, and magnesium.<sup>34-36</sup> High concentrations of fluoride and other ions have been detected in dentin adjacent to glass ionomer restorations.<sup>8,37</sup> Initial burst and long-term releases of fluoride from conventional and resin-modified glass ionomer cements have been reported.<sup>7,38,39</sup> Such fluoride demonstrated effectiveness has been demonstrated through the remineralization of caries lesion.<sup>40,41</sup> Sennou et al.<sup>42</sup> reported the diffusion of minerals, mainly fluoride, from glass ionomer cements into contacted dentin. They also reported the decalcifying effect of pre-treated cavity conditioner on dentin; result in having decalcified minerals (calcium and phosphate) in lesion. In our study, some released mineral ions from demineralization and from cavity conditioner pre-treatment, may remain in caries-affected dentin lesion and favor re-calcification. Re-growth of residual minerals enhanced by fluoride may contribute to remineralization and increasing of hardness in the lesion.<sup>43</sup>

Rate of remineralization depends not only on residual minerals in lesion but also on concentration of fluoride in that area.<sup>43,44</sup> The amount of fluoride that is required for remineralization of caries-affected dentin has not been reported. The content of fluoride in restorative materials should, however, be as high as possible and the release should be as great as possible. In our study, nanohardness changes at day 7 in Fuji IX GP group, and at every measured distance, were more than that of Fuji II LC group. This may be the result of higher fluoride and other mineral ions released from Fuji IX GP than Fuji II LC in this study condition. Comparative study data of fluoride release between these two cements were not clarified.<sup>45,46</sup> Regarding composition, setting reaction and physical properties, conventional glass ionomer cements were expected to have a higher potential for mineral release than resin-modified glass ionomer cements.<sup>47,48</sup>

Several previous studies reported higher fluoride release from convention glass ionomer cements than resin-modified glass ionomer cements, especially in the first week.<sup>39,41,49</sup> In our study, apart from fluoride, other mineral ions and environmental condition might initiate and promote remineralization of caries-affected dentin.

Significant increases of nanohardness of caries-affected dentin was observed in restored specimens of both groups, after 30 days of restoration. Nanohardness of caries-affected dentin adjacent to Fuji IX GP was significant higher than that of day 1, but was not significant higher than that of day 7. However, the nanohardness values at day 30 have not close to that of normal dentin. In the Fuji II LC group, only nanohardness at 20 µm from the cavity was significant higher than that of day 1. These data suggest the effectiveness of both materials on increasing hardness or remineralization of caries-affected dentin. Fuji IX GP seems to have more effectiveness than Fuji II LC due to the evident of more hardness-changed. However, differences between both groups regarding carious condition and mineral content of teeth that were used should be considered for the comparison. Further *in vitro* and *in vivo* studies should be designed to directly compare the effectiveness of both materials.

As for clinical implications, physiological remineralization of caries-affected dentin combines with physico-chemical remineralization that was generated by fluoride-releasing materials may provide benefits to this type of dentin. Hardness of caries-affected dentin under the combination of both types of remineralization may approach the level of normal dentin faster than having only one enhancer. In addition, the results of this study support the strategy of conservative intervention in preserving caries-affected dentin for restorative treatments.

## Conclusion

Conventional and resin-modified glass ionomer cements could promote some level of re-hardening on human caries-affected dentin.

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