Fluorescence Changes in Remineralized and Nonremineralized Enamel Adjacent to Glass Ionomer ART Restorations: An In Vitro Study

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ABSTRACT

Purpose: The purpose of this study was to evaluate fluorescence changes of remineralized and nonremineralized enamel margins adjacent to glass ionomer restorations during a pH cycling sequence.

Methods: One hundred permanent molar and premolar teeth were placed in a demineralizing solution for 3 days and restored with a glass ionomer restoration (simulating Atraumatic Restorative Treatment [ART]). Half were placed in a remin solution for 7 days to create a remineralization (remin) group. Specimens were randomly divided into 4 groups (N=25): (a) 2 remin groups; and (b) 2 nonremin groups. One half of the remin and nonremin group specimens were treated with a 5,000-ppm sodium fluoride solution during pH cycling with remin fluid and an acidic beverage over 20 days. Fluorescence changes were recorded with quantitative light fluorescence (QLF). Higher fluorescence values indicated less lesion porosity. Statistical comparisons between the groups over the 5 measurement sessions of cycling were performed using repeated measures of analysis of variance with a post-hoc test, paired-sample t test and 2-sample t tests (α=0.05).

Results: The remin groups experienced significantly less lesion porosity than the nonremin groups. Fluoride groups experienced less lesion porosity than the nonfluoride groups.

Conclusions: A brief period of remineralization and use of a prescription strength fluoridated rinse improved the enamel substrate surrounding glass ionomer restorations, resulting in less lesion porosity. (J Dent Child 2007;74:215-20)

KEYWORDS: RESTORATIVE TREATMENT, ATRAUMATIC, REMINERALIZATION, DEMINERALIZATION, ENAMEL, QUANTITATIVE LIGHT FLUORESCENCE

Concerns have surfaced about the effectiveness of caries prevention methods. Standard preventive recommendations include increased exposure to fluorides and a reduction in the number and frequency of cariogenic foods. Frequent use of acidic beverages throughout the day might increase the risk of caries.

A sucrose-rich environment, resulting from frequent ingestion of sugary foods or beverages, allows certain organisms to produce extracellular polysaccharides, which form a gelatinous material causing a diffusion-limiting barrier in the plaque.[1] The local environment then becomes anaerobic and acidic, conditions that favor tooth dissolution.[1] Lesion porosity increases as the acids demineralize the enamel-

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leaching calcium and phosphate ions with eventual collapse of tooth structure and cavitation.[2] The phenomenon of enamel degradation is complex (eg, at low pH levels), as seen with the frequent use of acidic beverages. Erosion is most likely the main factor in enamel degradation.[3]

Carbonated soft drinks could operate from both processes of demineralization—the transformation of bacterial byproducts and the acid in the beverage. Frequent use of acidic beverages might result in cavitated cervical lesions on the facial surfaces of teeth. Although these cervical lesions might be restored, little is known about effects of remineralization efforts and its measurement on the enamel surrounding these restorations, especially if acidic beverages continue to be consumed. Quantitative light fluorescence (QLF) offers a method of evaluating these enamel changes longitudinally by recording the enamel's change in fluorescence.

Amalgam alloy have traditionally been used to restore posterior carious lesions. Amalgam restorations were unesthetic, however, and did not strengthen surrounding tooth structure. Restoration of these posterior lesions with composite resin materials is more esthetic, but their longevity could be compromised if the lesions are not well isolated and not followed. Glass ionomer materials, bond directly to tooth structure, are not as technique sensitive as composite resin, and release fluoride. The atraumatic restorative treatment (ART) offers a method of restoring multiple lesions simultaneously.

ART was developed by field clinicians to address dental access problems in remote regions of the world without sophisticated dental equipment or electricity. There is no standard cavity preparation; sharp and irregular margins of the preparation are modified with hand instruments. Glass ionomer cements have been used as the preferred restorative material with the ART procedure, due in part to their anticariogenic behavior. These cements could contain 10% to 23% fluoride.[4]

The purpose of this in vitro study was to compare the mean fluorescence changes of enamel margins surrounding glass ionomer restorations in an environment simulating acidic beverage use. There were 2 research questions:

1. Would remineralization approaches make the enamel margins more resistant to demineralization during a pH-cycling period?
2. Does fluoride have any effect on remineralized and nonremineralized enamel during pH cycling?

METHODS

One hundred permanent molar and premolar teeth with intact, unrestored buccal surfaces were disinfected in Streck tissue fixative (Streck Laboratories, LaVista, Nebraska) for 2 weeks and then cleaned with hand scalers. Teeth were randomly divided into 4 groups (N=25 per group), color-coded, and numbered 1 through 25. A Class V cavity preparation (3 mm x 2 mm x 1.6 mm) was placed in the middle third of the buccal surface of each specimen using a sharp no. 330-carbide bur (Brasseler, Atlanta, Ga) in a high-speed handpiece (Kavo). Two layers of acid-resistant varnish (Classic Red Nailslicks, Novell Corporation, Hunt Valley, Md) were placed on each specimen, except for the preparation and a 1-mm perimeter of enamel around the preparation (window). All specimens were placed in a prerestoration demineralizing solution (2.20 mM calcium, 2.20 mM phosphate, 0.05 M acetic acid, pH 4.5) for 3 days to simulate the clinical appearance of demineralized enamel around the preparation (ART). They were then rinsed with deionized water. Preparations were conditioned with GC cavity conditioner (20% polyacrylic acid, GC Corporation, Tokyo, Japan) for 10 seconds and then rinsed with water. Fuji IX GP Fast (GC America Inc., Alsip, IL, USA) restorative material was placed into the preparation in a single layer using digital pressure, simulating ART.

Groups 3 and 4, which were restored with glass ionomer restorations, were placed in remin fluid (20 mM sodium bicarbonate, 3 mM sodium phosphate basic, 1mM calcium carbonate dihydrate) for 1 week (precycling remineralization). Specimens of groups 1 and 2, meanwhile, remained at room temperature (in 100% humidity) for 1 week. Groups 1 and 2 specimens were restored after specimens from groups 3 and 4 completed 7 days of remineralization. All groups started the 20-day pH cycling sequence simultaneously.

The 20-day pH cycling sequence was:

1. 4 hours in an acidic carbonated solution (Mountain Dew, PepsiCo, Inc, Purchase, NY)/groups 1 to 4;
2. 4 hours in a remin fluid/groups 1 to 4;
3. 4 minutes in a 5,000-ppm NaF solution/groups 1 and 4;
4. overnight in a remin fluid/groups 1 to 4.

Remin fluid was prepared daily, and the sodium fluoride solution was replaced every 5 days. The acidic carbonated solution was replaced daily.

A QLF system, which consisted of a clinical camera (Inspektor Quantitative Light-Induced Fluorescence System, v. 3.0.0.35, Inspektor Research Systems, Amsterdam, The Netherlands) connected to a PC (model no. VX920, Gateway, Inc, Irvine, Calif) was used for lesion analysis. QLF measurements were recorded for 8 periods listed in Table 1, using standardized drying and image capture protocols by 2 investigators. Fluorescence change (ΔF) was defined as the loss between actual and reconstructed fluorescence and was depicted as a negative value. Smaller negative values indicated less lesion porosity.

Table 1. Quantitative Light Fluorescence Measurement Sessions

Session No.	Step name	Groups
1	No treatment	1-4
2	Prerestoration demineralization	1-4
3	Restoration (remin groups)	3, 4
4	Baseline-start of cycling Restoration (nonremin groups) Precycling remineralization (remin groups)	1-4
5	Day 5 pH cycling	1-4
6	Day 10 pH cycling	1-4
7	Day 16 pH cycling	1-4
8	Day 20 pH cycling	1-4

For QLF analysis, standardized drying of each specimen consisted of:

1. placing a drop of deionized water on the buccal surface;
2. blotting with tissue paper; and
3. drying for 5 seconds with compressed air at its lowest setting.

Image capture occurred immediately after drying.

Comparisons between groups 1 to 4 over the 5 measurement sessions (sessions 4-8) during pH cycling were performed using repeated measures ANOVA with a post-hoc test (Table 2). 2-sample t test was used to compare fluorescence differences between sessions 1 and 3 and sessions 3 and 4. Paired t tests compared fluorescence differences between any 2 measurement sessions within each group. The statistical level of significance was set at $P<.05$. The mean fluorescence changes (ΔF) were analyzed at the 5% threshold level, similar to QLF evaluations in other studies.[5]

In addition, a few specimens were selected for sectioning for polarized light and scanning electron microscopy. A descriptive evaluation was made of these specimens.

Table 2. Treatment Group Mean Fluorescence (ΔF) Values

Precycling	Group 1	Group 2	Group 3	Group 4
No treatment	-2.48±3.91	-3.13±3.41	-2.19±3.82	-3.08±6.79
Prerestoration demin	-9.19±4.12	-8.75±2.66	-10.25±5.92	-9.45±3.55
Restoration	-13.95±5.95	-12.11±6.47	-13.23±8.93	-10.72±7.26
pH cycling	**Nonremin with fluoride**	**Nonremin without fluoride**	**Remin without fluoride**	**Remin with fluoride**
Baseline Precycling remin for groups 3 and 4	-13.95±5.95 A	-12.11±6.47 A	-11.21±7.59 A	-8.33±5.83 A
Day 5	-9.73±3.39 B	-7.66±5.29 B	-7.46±6.09 AB	-3.56±4.04 B
Day 10	-7.71±5.30 BC	-6.91±5.39 B	-7.30±4.89 AB	-3.35±4.20 B
Day 16	-6.73±4.85 BC	-5.80±4.90 B	-5.83±5.56 B	-2.62±3.61 B
Day 20	-5.13±4.52 C	-5.19±5.14 B	-3.86±4.31 B	-2.89±3.99 B

RESULTS

Specimens unresponsive to precycling demineralization (ΔF=0) were removed from further statistical analysis. The total number of specimens used in the final analyses is presented in Table 3.

Fluorescence values were less after specimens were placed in a demineralization solution for 3 days ($P<.0001$), which indicated there was an increase in lesion porosity (Table 2). Comparisons of fluorescence differences among groups revealed no statistically significant results ($P=.93$).

Table 3. Group labels and number of specimens used for analyses

Group	Label	Number of specimens
1	Non-remin with fluoride	23
2	Non-remin without fluoride	25
3	Remin without fluoride	24
4	Remin with fluoride	21

At the start of pH cycling, the only statistically significant difference was found between the designated fluoride groups (3 and 4; $P=.0095$). The data revealed that group 4 had significantly less lesion porosity (-8.33±5.83) than the nonremin with fluoride group (-13.95±5.95). This difference showed the effect of the precycling remineralization treatment in decreasing lesion porosity.

A 1-way ANOVA with repeated measures was conducted to evaluate the progression of pH cycling for each group (Table 2). There was a relative increase in lesion porosity from the start of pH cycling to day 20 for all groups except group 4, which received fluoride. From day 16 to 20, there was a decrease in -ΔF values, but this was not statistically significant. groups 1 and 3 had significant differences throughout cycling. In groups 2 and 4, however, the baseline was significantly different from days 5, 10, 16, and 20. Tukey post-hoc test results are presented in Table 2.

Based on the 2-way ANOVA with repeated measures, there were no significant interactions between group type and measurement session. Furthermore, this result suggested that differences in fluorescence changes between groups were consistent over the measurement sessions during pH cycling. The main effect of the group was not significant ($P=.13$), although the measurement session showed a significant main effect ($P<.0001$). The Tukey post-hoc test revealed that the values of fluorescence change between days 5 to 20 were significantly higher (less lesion porosity) than values at baseline and that the values at days 16 and 20 were significantly higher than day 5.

Several specimens from each group had pitting and voids after a few days of pH cycling, which increased throughout the cycling period. Figure 1 shows the restoration pitting. In addition, by the end of the 20-day period, several specimens had large cavitated lesions on the cervical margins.

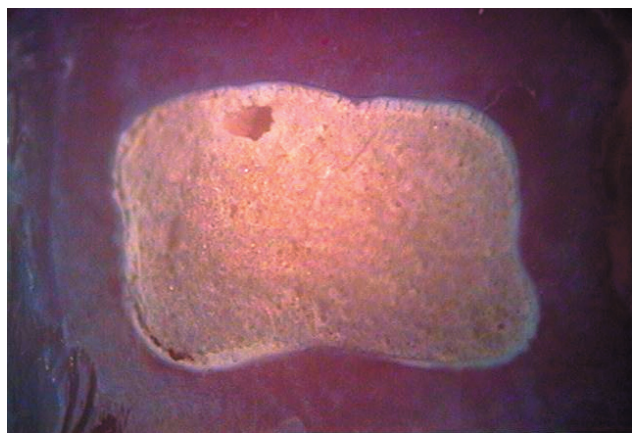

Figure 1. Specimen Showing Surface Pitting of Restorative Material

DISCUSSION

This study evaluated the fluorescence changes in remineralized and nonremineralized enamel margins during pH cycling surrounding glass ionomer restorations. The variation in fluorescence throughout the pH cycling was consistent across QLF measurement sessions and among groups. This could be due to the inherent nature of the specimens, or the effect of treatment. The results of this in vitro study demonstrated that a remineralization period before pH cycling did influence fluorescence values after pH cycling by decreasing lesion porosity. Remineralized specimens had less lesion porosity. Fluoride use also decreased lesion porosity, but not to the same extent.

The least lesion porosity was recorded in group 4 (Table 3). There were many factors associated with these results. Group 4 had a 7-day precycling remineralization period, exposure to fluoride from the glass ionomer restorative material[5-7], and exposure to fluoride from the fluoride rinse[6,8] throughout the 20-day cycling period.

During remineralization, tooth minerals—predominately calcium and phosphate—are deposited in tooth structure.[9] The surface zone is also preserved by the presence of fluoride.[10] Fluoride, added during this process, might replace the hydroxyl group of the hydroxyapatite and create fluorapatite, which is more resistant to acid dissolution.[9] The combined effect of the restorative material, 7-day remineralization period, remin fluid, and fluoride rinse could have prepared group 4 to resist lesion porosity. A 14-day pilot study demonstrated that Fuji IX GP Fast released, recharged, and rereleased fluoride when placed in deionized water. Topically applied fluoride might change the enamel surface morphology and facilitate improvement in lesion porosity.[8]

The enamel margins of group 4 specimens had less porosity than those of group 1 (Table 3). This could be the result of the recharge of the glass ionomer material and the release of fluoride to adjacent tooth structure. Some authors determined that glass ionomer restorative materials could release, recharge, and rerelease fluoride and the recharge was 6 to 10 times greater.[5]

The fluorescence differences among groups 3 and 4 (the remin groups) in the present study, however, were greater than those for groups 1 and 2 (fluoride group). This finding implies that the fluoride recharge might not be better than a precycling remineralization period.

In this study, group 2 had the greatest lesion porosity of any group. Remineralization did occur, however, not demineralization. One possible explanation was the fluoride in the acidic carbonated beverage. A study by Heilman *et al*[11] found a mean fluoride level of 0.72 ppm in fluoride assays of 332 carbonated soft drinks, with a range of 0.02 ppm to 1.22 ppm fluoride for PepsiCo, Inc. products. The main determinant of fluoride levels was the water used at the production site. The amount of fluoride in the beverage closely paralleled the fluoride concentration in the water of the bottling facility.[11] The beneficial contribution of the fluoride content in an acidic carbonated beverage to decrease lesion porosity requires further study.

QLF is affected by staining, positioning, moisture, and ambient lighting.[12] This study used standardized positioning, drying, and lighting protocols in the QLF measurement sessions to improve reproducibility. Pretty et al[13] studied 3 methods of drying specimens for QLF analysis bench drying, cotton wool roll drying, and compressed air with deionized water and saliva. They found compressed air to be the most effective system for reliable readings. Angmar-Mansson and ten Bosch[14] mentioned that dehydration in vitro decreased fluorescence readings by a factor of 0.10 to 0.15. Amaechi and Higham[15] recommended drying with a cotton-wool roll rather than a 3-way compressed air syringe for better control. The protocols for drying, positioning, and lighting used in this study, provided adequate controls to improve reproducibility. The high correlation found with transverse microradiography[12,16-18] permitted QLF to be a viable diagnostic option for this study.

The rationale for limiting analysis to the occlusal enamel of the Class V restoration and preparations to the middle third of the facial surface were based on the thickness of the enamel, QLF sensitivity in detecting incipient carious lesions, and results of a pilot study. A pilot study found less variability on the occlusal aspect as oposed to the cervical aspect.

ART acceptance in the general dental community is unknown. The availability of anesthesia, rotary instruments, and encapsulated materials simplifies caries removal and restoration placement. Future studies might investigate ART in general dental practice.

If a restorative material is recommended for a definitive restoration, there should be evidence to confirm that it has adequate physical properties and longevity. Burke et al[19] found general dental practitioners using glass ionomer materials for high caries patients or those with poor oral hygiene. Mjor and Gordan[20] cited marginal breakdown and loss of restorative material as the most common reasons to replace glass ionomer restorations. It is likely that special conditions such as an assessment of high caries risk from frequent use of acidic carbonated beverages prohibit the material's use as a definitive restoration in these individuals.

In this study's methodology, the glass ionomer restorations of groups 3 and 4 were placed 1 week earlier than the glass ionomer restorations of groups 1 and 2. This 1-week difference might have contributed to the decrease in enamel porosity exhibited by groups 3 and 4 during pH cycling. The 1-week difference in restoration maturity might have also contributed to restoration pitting, as shown in Figure 1. These observations require further study.

The overall content of fluoride available could have confounded the results, both in its use in the processing of an acidic carbonated beverage and release of fluoride from the glass ionomer restoration. The beverage's fluoride level depended upon the fluoride concentration of the water at the production site.[11]

Four hours of continuous exposure to an acidic carbonated beverage and 4 minutes of continuous exposure to a 5,000-ppm fluoridated solution might not be practical in a real-life situation. Most likely, there would be shorter,

more frequent exposures, which could result in less severe demineralization. Intermittent exposures to a carbonated beverage might allow the natural buffering capacity of human saliva to initiate more periods for remineralization, which is a much slower process. A study by Forshee and Story[21] found a modest association between carbonated soft drink consumption and DMFS (decayed, missing, and filled surfaces) for older adults and little or no association for young adults and adolescents. Marshall et al,[22] however, suggested that an increase in soft drink consumption for young children might increase children's dental caries rates. Indeed, oral physiology, frequency of ingestion, and exposure to fluoridation are a few factors that influence the pattern of remineralization and demineralization.[3]

Pitting of glass ionomer restorative material during the duration of this in vitro study was most likely accelerated by the 4-hour exposure to an acidic carbonated beverage. In a real-life situation, the buffering capacity of human saliva might help reduce pitting by bathing the surface with valuable ions. Further study is required to evaluate intermittent vs. continuous exposure of tooth surfaces surrounding glass ionomer restorative material to an acidic carbonated beverage and topical fluoride.

This was an in vitro study, so caution should be exercised in extrapolating these results to a clinical situation. This study, however, suggests that restoring Class V lesions with glass ionomer restorations and doing as much preventive care (dietary changes, reduction in cariogenic snacks, use of prescription strength fluoride rinse), even for a brief period, might render tooth structure more resistant to further cariogenic challenges presented by the frequent use of acidic carbonated beverages.

CONCLUSIONS

Based on this study's results, the following conclusions can be made:

1. Remineralized enamel recorded higher fluorescence values than nonremineralized enamel during a 20-day cycling period with prolonged use of an acidic carbonated beverage.
2. The QLF system was able to monitor changes in vitro using protocols that reduced errors in positioning and lighting.
3. The dissolution of glass ionomer restorative material during pH cycling suggests that its physical properties might limit its use as a definitive restoration.
4. Other studies are required to evaluate these findings in vitro, in situ, and in vivo.

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