TIME-DEPENDENT FLUORIDE UPTAKE INTO DENTIN FROM A RESIN-MODIFIED GLASS IONOMER

by

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BACKGROUND AND SIGNIFICANCE

Introduction

In a study nearly 70 years ago, Volker et al. (1944) found low caries prevalence associated with silicate rather than amalgam restorations.[1] It was suggested that fluoride was released from the silicates and taken up by enamel at restoration margins.[2] Since Volker's research, fluoride release from dental materials and uptake into tooth structure has been studied extensively and today is an important aspect of clinical dentistry. Although it is now universally recognized that fluoride can provide protection to teeth from caries, the exact mechanism is not evident. In fact, there are many possible ways that fluoride may affect the caries process and demineralization. The primary mechanisms by which fluoride prevents caries are: 1) resistance to demineralization, 2) facilitation of remineralization, and 3) by effecting metabolism of cariogenic bacteria.[3] These mechanisms are both chemical and antimicrobial in nature. The chemical mechanisms of fluoride's actions involve incorporation into unerupted teeth as well as the enhancement of surface mineralization and remineralization with both hydroxy- and fluorapatite. Fluorapatite, a compound in which fluorine is substituted for hydroxyl groups in the chemical formula $Ca_{10}(PO_4)_6(OH)_2$, provides approximately 20% less dissolution of calcium ions in acid compared to hydroxyapaptite. Interestingly though, it has been reported that the incorporation of fluorine into hydroxyapatite does not result in the complete elimination of OH^-; fluorine generally replaces only one OH^- group, forming a fluorohydroxyapatite compound.[4] The critical pH at which hydroxyapatite dissolutes is 5.5, while that of pure fluorapatite is 4.5. Thus, fluoride incorporation into teeth at low levels can help prevent demineralization. The antimicrobial effects of fluoride generally involve bacterial enzyme inhibition which is enhanced by lower pH environments.[5]

Fluoride uptake by teeth is one of the criteria for evaluation of the cariostatic properties of a restorative material.[6] Fluoridated dental materials may not only release fluoride into tooth structures, but can increase the levels in saliva and adjacent plaque to a point that inhibits carious activity. With fluoride concentrations as low as 0.02 to 0.06 ppm, it has been shown that enamel

dissolution can be reduced when placed in a demineralizing solution. Similar results can occur with dentin. In fact, fluoride uptake in dentin is enhanced by its greater porosity, higher water content, lower crystalline content, and smaller sizes of crystals.[2] However, while dentin's capacity for fluoride uptake is much higher than enamel, higher concentrations of fluoride are needed to prevent demineralization and enhance remineralization.[8]

In vitro and in vivo research studies have established that glass ionomer restoratives have significant fluoride release and are effective in preventing recurrent decay and demineralization. The typical element composition of the glass particles within a glass ionomer cement includes silicon, aluminum, strontium, calcium, and fluorine, in various amounts. Conventional glass ionomer cements set through an acid-base reaction. Water is essential to this reaction, in which polyacrylic acid reacts with the basic fluoro-aluminosilicate glass. Unreacted glass helps reinforce the material. It is from these glass particles that the high fluoride concentrations are found. The polyacid matrix of the glass ionomer is one of the routes through which the fluoride ions may diffuse. There are two different stages of setting within a glass ionomer. The initial setting consists of cross linking of the polyacid through Ca, Al and other ions. Maturation of the material takes longer, and includes an increase in bound water within the matrix.[8] There are two mechanisms for fluoride release from a material: surface dissolution of fluoride ions and bulk diffusion of fluoride from within the material. Surface dissolution may be most evident in the early stages of restoration maturation and at initial setting. Fluoride release may even be displayed at the surface of glass ionomers after "recharging" them using a high-concentration fluoride rinse, gel, or toothpaste. Bulk diffusion within the material is a very slow process, which may result in low levels of fluoride release over long periods of time. However, it has been suggested that fluoride release may occur partly though diffusion in the glass ionomer cement pores and cracks, which can speed up the process.[9]

Over the past decade, new materials with fluoride have been developed, including hybrid ionomers (also called resin-modified glass ionomers, or RMGICs), compomers (also called polyacid-modified resin composites), and fluoride-containing composite resin. Along with the many new materials available to dentists come questions regarding their fluoride release and ability to inhibit demineralization. While many studies have demonstrated significant amounts of fluoride release from glass ionomers and hybrid ionomers, compomer and composite restoratives have been shown to have significantly less fluoride release over time. This is

understandable, since the initiation and progression of the polyacrylic acid-basic glass setting reaction of glass ionomers is essential for significant amounts of fluoride release. Because most compomers and fluoride-containing composites set primarily upon photoinitiation, they would be expected to have relatively low fluoride release.[1] In fact, the transfer of fluoride from compomer and composite restorations directly into dentin is thought to be through the slow process of water sorption or restoration margin leakage, which leaches fluoride from the glass particles within the material.

Introduced in the late 1980s, hybrid ionomers are similar to glass ionomers, but differ in that acidic and polymerizable polymers are present and set through both acid/base and polymerization reactions.[11] Advantages of RMGICs include longer working times and controlled setting times, fast development of strength and lower sensitivity to environmental moisture changes. They can also be finished and polished immediately after setting.[3] While glass ionomers are particularly moisture sensitive and manufacturers often recommend covering restorations with a surface sealant, RMGICs often are placed without such sealants. Although glass ionomers and hybrid ionomers possess cariostatic qualities, low tensile strength, brittleness, and low resistance to wear preclude these materials from being used in some load-bearing long-term situations clinically.[12] Nevertheless, such materials are still an important part of restorative dentistry and caries prevention. Many of these materials are used by today's dental clinicians for permanent restorations or even bases and liners, providing protection to pulpal tissues. Dentists often may use these materials anticipating that they will release fluoride and provide protection from recurrent decay or remineralize decalcified tooth structure.

Analysis of fluoride release

Research has shown that large amounts of fluoride ions are released during the early life of a glass ionomer cement, but the release gradually decreases over time, unless the material is recharged.[13] For example, Miranda et al. (2002) reported a high initial rate of fluoride release, followed by a rapid decrease after 24-48 hours, then reaching a constant release level, unless recharged.[14] Additional studies have investigated fluoride release from different materials immersed in an aqueous environment. The most widely used method to measure fluoride release is through the use of an ion-selective (or ion-specific) electrode. While this chemical analysis

method can show differences in fluoride release between different materials, it does not accurately predict how the materials will interact with dentin. In these studies, researchers have employed fluoride ion-selective electrodes to measure the amount of fluoride released in an aqueous environment. Alternatively, chemical or physical methods have been employed to destroy tooth specimens and then ion-selective electrodes used to measure the amount of fluoride released. Unfortunately, few studies have attempted to correlate the measured fluoride release in an aqueous environment with the fluoride released directly into dentin. Such findings are of limited value, as they do not report the depth of fluoride uptake into tooth structure. In fact, studies on the efficacy of fluoride release from dental materials are innumerable, but the amount and depth of fluoride incorporation into dentin are not well-defined.[2] Furthermore, the concentration and depth of fluoride penetration from glass ionomer-based restoratives has not been adequately studied as a function of time for restorative materials in contact with dentin.

In addition to ion-selective electrode measurements, other methods to measure fluoride include gas or ion chromatography and abrasion biopsy (in conjunction with ion-selective electrode analysis). Of those studies that have reported on fluoride uptake directly into dentin and enamel from dental materials, most have employed one of these methods. All these methods involve destructive testing where the original shape of the specimen is destroyed during measurements.[6] Only the abrasion biopsy method determines the amount of fluoride uptake at sequential depth increments, though the increments are often large. The physical measurement of fluoride using electron microprobe analysis does not destroy the specimen during testing. This method is useful in that fluoride uptake within tooth structure may be mapped two-dimensionally to visualize the spatial distribution of fluoride from the tooth-restorative interface. Furthermore, the specimen is preserved intact for additional analyses such as resistance to demineralization

Electron microprobes are very powerful tools for the microanalysis of materials. The first rudimentary electron-probe analyzer was likely that built by Starke in 1898.[15] Progressively, advances in the use of X-rays for element identification were developed. In 1949, the first commercial electron microprobe was developed by Castaing.[16] Electron microprobes work by generating secondary X-rays in a sample through electron bombardment. The wavelengths and intensities of the X-rays produced are used to identify the elements and their relative concentrations. With a very fine electron beam, minute areas within a specimen can be

analyzed.[15] There are two types of microprobe settings: EDS (energy-dispersive spectrometer) or EDX, and WDS (wavelength-dispersive spectrometer) or WDX. EDS identifies elements by energy, using a solid-state detector that discriminates between energies of incoming photons.[17] The EDS system can identify and quantify elements with atomic numbers greater than 11, although certain modifications have recently allowed elemental analysis for atomic numbers ≥ 4 (Beryllium). WDS identifies elements by wavelength, using a diffracting crystal to isolate characteristic X-ray peaks.[17] WDS systems can identify multiple elements simultaneously, including elements with atomic numbers <11. As fluorine has an atomic number of 9, and often multiple elements must be identified at the same time during the analyses, WDS would be the system of choice for analyzing tooth structure. Additionally, trace element analysis (weight % < 10,000 ppm for light elements such as fluorine) must be determined using WDS.[16] Other features of WDS make it ideal for measuring fluoride in dentin. For instance, the higher peak to background ratio obtained using WDS is a big advantage for low concentrations such as fluorine[15]; WDS yields higher peak to background ratios than EDS, thus allowing for lower detection limits. Furthermore, superior resolution makes WDS a more feasible option for identifying the fluorine peaks in the X-ray spectrum, as the fluorine Kα line (18.32Å) appears close to the Kα line of phosphate (18.45 Å). In addition to basic WDS analysis, quantitative analysis can be conducted using a specimen of known composition as a standard. Corrections for details such as background noise can be accounted for through subtraction.

There are some limitations to WDS electron microprobe analysis in measuring small concentrations of fluoride in dentin. For instance, instrument settings such as voltage, amperage, beam focus and analysis time per point must be carefully adjusted for biological specimens such as dentin. A delicate balance must be achieved to avoid overheating and destroying the area of analysis while still providing enough energy to result in adequate X-ray peak counts for fluorine. Since detection limits are dependent on the ratio of peak counts to background levels, the detection limits for biologic specimens may be higher than desired. Even with the best calibrated settings, the detection limit for WDS of fluorine within dentin may be within the range of 200-500 ppm. Thus, while the electron microprobe has many advantages over fluoride detection methods such as the F ion-selective electrode, the detection limit for fluorine within dentin is significantly higher.

Effectiveness of fluoride release

There are two main approaches for evaluating fluoride uptake into dentin. One approach is to measure fluoride directly from the tooth through chemical or physical methods, such as electron microprobe analysis. The other approach is to measure fluoride incorporation into dentin indirectly by assessing the inhibition of demineralization, acid resistance, or resistance to carious attack. Microradiography, polarized light microscopy, or hardness measurements may be employed for this assessment. While direct measurement of fluoride within tooth structure is an excellent method of evaluation, it does not demonstrate the cariostatic properties of the tooth. The question of exactly how much fluoride is needed to inhibit demineralization within dentin has still not been answered completely by researchers. The amount of demineralization obviously depends on the intensity of the acid attack as well as the type of material. As previously discussed, due to its porous structure dentin is more prone to demineralization than enamel. Another question that needs to be answered is whether a correlation can be determined between absolute fluoride amounts within dentin and resistance to acid attack. Numerous studies have focused on the effectiveness of fluoride in inhibiting demineralization and carious attack on dentin, but none have correlated the measured amount of fluoride to the ability to inhibit demineralization.

Innumerable methods for measuring resistance to demineralization have been utilized by researchers. Unfortunately, there appears to be no consistent standard in the sequence of either creating artificial carious lesions or subjecting intact tooth specimens to demineralization protocols. Included in the multitude of different demineralization solutions are various concentrations of lactic acid, acetic acid, citric acid, phosphoric acid, and glucose and S. mutans-infused broths, some of which are buffered. pH values range from less than 1 to over 5, with most pH values around 4.5. Immersion times range from minutes to weeks, depending on the demineralization sequence. However, for basic research purposes a simple and repeatable solution and process should be utilized. Since in vivo carious attack has been shown to arise from the bacterial formation of lactic acid and acetic acid, it seems reasonable to utilize a solution based upon acetic acid.

Specific objectives and hypotheses

<u>**Statement of Hypotheses:**</u>

Ho_1:   For Fuji II LC there will be no statistically significant differences between fluoride
           uptake and diffusion profiles in dentin of specimens matured for 1, 15, and 30 days.

Ha_1:   For Fuji II LC there will be statistically significant differences between fluoride uptake
           and diffusion profiles in dentin of specimens matured for 1, 15, and 30 days.

Ho_2:   There will be no statistically significant differences between the microhardness
           measurements of demineralized dentin adjacent to Fuji II LC restorations matured for 1,
           15, and 30 days.

Ha_2:   There will be a statistically significant difference between the microhardness
           measurements of demineralized dentin adjacent to Fuji II LC restorations matured for 1,
           15, and 30 days.

<u>**Primary Objective:**</u>

The primary objective of the proposed research is to use a non-destructive method to measure the
amount of fluoride uptake and the depth of penetration into dentin from a resin-modified glass
ionomer restorative material (Fuji II LC capsule). The experimental technique used to determine
the fluoride content will be electron microprobe analysis. While this method has been available
for decades, only recently has the instrumentation evolved sufficiently to measure elements such
as fluorine precisely and accurately, with minimal background noise. In particular, WDS
(Wavelength Dispersive Spectroscopy) is the method of choice for the analysis, as it may
measure elements with relatively small atomic weights, such as fluorine. The tooth-restorative
material interface will be examined at the axial wall of Class V cavity preparations in non-
carious teeth at different time intervals after initial placement. A linear analysis of the amount of

fluoride within the dentin will be determined for each specimen to create a diffusion profile for each time interval.

<u>Secondary Objectives:</u>

1) Demonstrate repeatable and reliable methods for the determination of fluoride concentrations in dentin.

2) Subject each electron microprobe specimen to a demineralization challenge and measure the microhardness of the dentin along a line oriented perpendicular to the axial wall of the tooth-restorative interface.

3) Determine if there is a measurable correlation between fluoride uptake in dentin and higher microhardness of the surfaces after being subjected to a demineralizing challenge.

REVIEW OF THE LITERATURE

Fluoride ion-specific electrode studies

For many years a common method for fluoride determination was to use a colorimetric procedure. However, with the fluoride ion activity electrode developed by Frant and Ross (1966), fluoride measurement became simpler.[18] An evaluation of the effectiveness of a few different methods of fluoride analysis was conducted by Retief et al. (1985).[19] In the study, a F-specific electrode method was compared to gas chromatography as well as a microanalytical technique involving a modification of the F-specific electrode.

For fluoride analysis with a *standard combination F electrode*, standard fluoride solutions with different ppm fluoride ion were prepared and diluted with equal volumes of distilled water. Measurements for a calibration curve were prepared with a F electrode coupled to a model 801A digital ionalyzer (Orion). For fluoride analysis with a *modified F electrode*, the apparatus consisted of a F electrode and a miniature calomel reference electrode (Fisher Scientific) coupled to an Orion digital ionalyzer. Calibration curves with the same F concentrations for the standard F electrode combination were used. Recovery of various amounts of F added to a standard F solution (1.25 ng F/5 μL solution) was evaluated with the modified F electrode apparatus. Ten analyzes were performed for each solution. For fluoride analysis using *gas chromatography*, the technique was employed that is based upon the hydrolysis of trimethylchlorosilane to the silanol which reacts selectively with F to form trimethylfluorosilane.[19] The recovery of various amounts of F added to a standard solution of 0.5 ng F/2 μL injection volume was evaluated. Ten analyzes were performed for each solution.

Enamel samples from 20 extracted maxillary molars were obtained by using a carbide bur in a slow-speed handpiece and dissolved in perchloric acid. Various dilutions and preparations were made for analysis with one of the three techniques. Results of the measurements showed that with the standard F electrode, analysis of a 0.95 ppm F solution yielded 0.965 ± 0.013 ppm with 10 separate measurements. With the modified F electrode, the recovery of the various solutions demonstrated over 92% success except with lower levels of F. With the gas chromatography method, success was from 82-98%, with lower recovery percentages accompanying lower F concentrations. When comparing the enamel samples from the 20

different maxillary molars, there were minute, insignificant differences between the three techniques. In essence, all three methods showed similar capability to measure fluoride concentrations. The authors demonstrated that a modified F electrode method is no more sensitive than the standard electrode method. However, the technique, according to the authors, is faster and less technique-dependent. According to the data, it appears that all of these methods are much more sensitive to minute fluoride concentrations than electron microprobe analysis. The drawback to all these techniques is that the tooth specimen must be dissolved in each case, with only the overall amount of fluoride determined. Despite some of the limitations of the ion-selective electrode, it is a relatively simple instrument to use and has been utilized frequently in research for measuring fluoride. Therefore, it is important to evaluate some studies that employed this technique to measure fluoride release.

In a study by Yip et al. (2000), differences in fluoride release between compomers and resin-modified glass ionomers were measured using an ion-selective electrode.[9] Using Dyract (a compomer), Fuji II LC, Photac-Fil, and Vitremer (RMGICs), five specimens per material were prepared to dimensions of 30 mm in diameter and 2.7 mm in thickness. Specimens were placed in vials of deionized water at 37°C, which was replaced weekly and fluoride ion measurements done at day 1, 7, 30, and every 28 days after day one, over a 253 day period. A fluoride ion-selective electrode was used for measurements after 1 mL of solution was removed from the vials and mixed with buffer.

Results of the study showed that all the RMGICs had high initial release rates. However, the compomer Dyract had minimal release of fluoride throughout the testing period. After 84 days, the release rates of fluoride were statistically insignificant and after 253 days had plateaued. At day one, fluoride release was measured with the following order: Photac-Fil > Vitremer > Fuji II LC > Dyract, which had minimal fluoride release. At day 84 as well as day 253 the order of fluoride release was: Photac-Fil > Fuji II LC > Vitremer > Dyract. The main conclusions of the study were that the compomer (Dyract) released significantly lower amounts of fluoride immersed in deionized water compared to the other resin-modified glass ionomers. This result is typical of many studies on glass ionomer-based materials compared to compomers.

In another similar study measuring fluoride release from various materials, Aboush and Torabzadeh (1998) demonstrated release over 12 months from Fuji II LC, Photac-Fil Aplicap, and Vitremer, all resin-modified glass ionomers.[20] Also included were Fuji Cap II (a

conventional GI), Dyract, and Tetric (a fluoridated composite resin). For each material, five disc specimens measuring 7 mm in diameter and 2 mm thick were fabricated. Following setting of the materials, each specimen was suspended in 4 mL of deionized water at 37°C. After 24 hours, the solution was replaced. Two mL of each of the test solutions were added to 200 mL of 0.1 mol/L HCl and measured for fluoride content using an ion-selective electrode system. This procedure was continued daily for 30 days. After 30 days, specimens were removed only every 12 days, over the course of 3 months. Then for the next 8 months, the procedure was repeated every 27 days. In total, 86 measurements were made. Some of the results are shown below in Table 1.

Table 1. Fluoride Release from Various Restorative Materials.[20]

Material	Cumulative fluoride release ($\mu g/cm^2$)	Average fluoride release in the last 24 h (at 12 months)
Photac-Fil Aplicap	1154 ± 86	5.0 ± 0.8
Vitremer	593 ± 41	2.5 ± 0.3
Fuji II LC	580 ± 17	2.3 ± 0.2
Fuji Cap II	480 ± 42	1.3 ± 0.2
Dyract	87 ± 17	0.23 ± 0.02
Tetric	22 ± 2	0.21 ± 0.04

Adapted from Aboush and Torabzadeh (1998)

Fluoride release for all materials was highest over the first 24 hour period, quickly declining thereafter. All the materials reached constant fluoride release levels, with the resin-modified glass ionomer materials leveling out sooner than the conventional glass ionomer (Fuji Cap II). The compomer, Dyract, behaved mostly like the fluoride-containing composite (Tetric), with minimal release. In the resin-based materials, fluoride is most likely released through surface leaching as well as a very slow diffusion process. Photac-Fil applicap specimens consistently demonstrated the most fluoride release. Such a finding is not surprising, as many other studies have found similar results. The authors wisely suggest in the article that not only fluoride release from restorative materials, but also the prevention of microleakage at margins plays a part in preventing secondary decay. However, along with microleakage at margins may come some dissolution of glass ionomer materials, leading to increased fluoride release and inhibition of demineralization. In fact, in some research on compomers and fluoride-containing

composites and bonding agents, it is only those margin areas with microleakage that demonstrate any significant release of fluoride.

In another study using an ion-specific electrode, Weidlich et al. (2000) studied fluoride release from glass ionomers and composite resins.[21] Vitremer (RMGIC), Fuji II LC (RMGIC), Fuji IX (GIC), Chelon Fill (GIC), Heliomolar (composite resin with fluoride), and Z-100 (composite resin without fluoride) were all tested for the amount of fluoride release into artificial saliva. For each group, 8 specimens measuring 8 mm in diameter and 2 mm in thickness were prepared according to manufacturers' instructions. All specimens were kept in 37°C water for 24 hours and then placed in tubes containing 4 mL of artificial saliva. After another 24 hours, 5 of 8 specimens from each group were removed, immersed in 1,000 ppm fluoride solution for 1 minute, and then replaced in artificial saliva. This immersion in fluoridated solution was repeated daily for 25 days. Fluoride-ion specific electrodes were used to measure the artificial saliva tubes at 1, 2, 5, 10, 15, 20, and 25 days. Results of the testing showed that specimens not exposed to fluoride released the most fluoride during the first day. Subsequently only the RMGIC and GICs released fluoride, but at a decreased rate (see Table 2). With fluoride exposure, it appeared that the RMGICs and GICs did take up fluoride, and continued to release a relatively high amount (though slightly reduced) throughout the 25 days.

Table 2. Fluoride Release from Various Materials, Measured With a F Ion-Specific Electrode.[21]

Material	Fluoride Release ($\mu g\ F/cm^2$)					
	1 day	2 days	5 days	10 days	15 days	20 days
Vitremer	1.04	0.10	0.12	0.04	0.04	0.11
Fuji II LC	0.68	0.49	0.36	0.20	0.04	0.08
Fuji IX	0.52	0.09	0.17	0.43	0.00	0.00
Chelon Fill	0.63	0.20	0.34	0.45	0.00	0.04
Heliomolar	0.16	0.00	0.00	0.03	0.00	0.00
Z-100	0.00	0.02	0.00	0.00	0.01	0.00

Adapted from Weidlich et al. (2000)

The results from this study not only indicate that both GICs and RMGICs release fluoride in measurable amounts, but again demonstrated that composite resins release essentially no fluoride; Heliomolar released fluoride, but only on day one. Composite resins with fluoride would be expected to release little or no fluoride into the dentin since there will be a barrier—an adhesive layer—between the composite resin and the dentin. An interesting observation from

the authors of this article was that fluoride release in artificial saliva is lower than in deionized water.[21] According to the authors, this is because a coating forms on GICs when exposed to saliva (or artificial saliva). Thus, for research purposes to enhance any differences between materials, deionized water may provide a better medium for fluoride release

Another study comparing fluoride release from various materials was published by Miranda et al. (2002). Using an ion-selective electrode analysis, fluoride release was measured from Vitremer (RMGIC), Heliomolar (fluoride-containing composite), and Z-100 (non-fluoridated composite) restoratives.[14] Ten discs of each material, measuring 6 mm in diameter and 2 mm in thickness were prepared. Orthodontic wire was used to suspend the samples in water. All specimens were light cured in three different positions for 40 seconds. Half of the specimens were then covered with one layer of Scotchbond MP Plus adhesive, and light cured for 20 seconds each. All specimens were suspended in 37°C water for 24 hours and transferred to a flask of artificial saliva for 20 days, changing the solution every day. Fluoride release was measured from 1-20 days using a fluoride ion-specific electrode. Results of the testing showed that fluoride release from Z-100 with and without adhesive was nonexistent. Furthermore, Heliomolar with adhesive specimens did not release any fluoride. At 24 hours, the Vitremer specimens released the most fluoride (0.89 ± 0.26 μg F/cm^2), followed by the Vitremer specimens covered with adhesive (0.22 ± 0.14 μg F/cm^2). Heliomolar without adhesive released fluoride, but at a significantly lower level (0.11 ± 0.08 μg F/cm^2). After five days, the Vitremer specimens had a significant decrease in fluoride release. This significant decrease was seen after just one day in the Vitremer + adhesive group and the uncoated Heliomolar specimens. This study showed that adhesive greatly reduced the release of fluoride from RMGIC and made fluoride release undetectable from a fluoride-containing composite. The fluoride-containing composite Heliomolar was only effective releasing minute amounts of fluoride when an adhesive was not used. The applicability of this study to clinical situations may be minimal, as conditions were dissimilar to in vivo situations. Supposing that composite materials should and will be used with bonding agents, it is expected that even fluoride-containing composites will not release detectable amounts of fluoride into dentin along the tooth-restorative interface. Furthermore, RMGICs are generally used without bonding agents which would reduce the fluoride release from the material.

The effect of maturation and time

The phenomenon of glass ionomer maturation is important, as RMGICs have a portion of setting induced by visible light, which greatly speeds up the setting process at a time chosen by the clinician. Because of this faster setting and the resin component of the material, some have questioned the short-term, as well as the long-term fluoride releasing ability of RMGICs. A study by Marks et al. (2000) reported on the effect of maturation of RMGICs and compomers on the fluoride release.[10] Ten specimens measuring 6 mm in diameter and 3 mm thick were fabricated for Vitrebond, Vitremer, GC Lining LC, Variglass (all hand-mixed), Dyract (no mixing) and PhotacBond (triturated). All specimens were dispensed into transparent molds and light cured according to manufacturers' instructions. Post-curing, specimens were either transferred into 25 mL ultra-pure water, or matured under 85% water vapor for 24 hours prior to transferring into the 25 mL water-filled flasks. All flasks were shaken in a water bath at 37°C, with the water renewed at 0.25, 1, 2, 3, 4, 7, 14, 21, 28, 56, 84, 112, 140, and 168 days. After buffering eluates, a fluoride ion-selective electrode was used to determine the fluoride concentrations within the solutions after each time interval.

Results of the experiments showed that the RMGICs released much more fluoride than compomers. Furthermore, for RMGICs, after 24 hours of maturation prior to immersion in water, specimens released much less fluoride than samples immersed immediately after set. This relationship continued over short-term and long-term (up to 168 days) testing. Such a difference was not seen with either Dyract or Variglass (both compomers). Overall, the amount of fluoride release by the various materials showed that that the RMGICs all released significantly more fluoride than the compomers. A comparison of the fluoride release between the different RMGICs showed the following trend: PhotacBond>Vitrebond>GC Lining LC>Vitremer after immediate immersion. With 24 hours maturation prior to immersion, the order was essentially the same. Interestingly, this study shows that even with similar materials and chemistries, different commercial RMGICs demonstrate significantly different amounts of fluoride release. As observed in the experiment, immediate immersion in an aqueous solution affects fluoride release in RMGICs, as the acid-base reaction is impacted by such immersion. While fluoride may be released in significantly higher amounts into solution when immediately immersed versus matured prior to immersion, the material's mechanical properties may be adversely

affected. This explains why many manufacturers suggest using a "glaze material" or a surface varnish over restorations to protect them from overexposure to moisture over the initial 24 hours.

While many studies have shown the fluoride release patterns of various materials over time in an aqueous environment, Tay and Braden (1988) went a step further, showing the relationship as a function of time.[22] In the article, fluoride ion diffusion from glass ionomer cements was measured. Six glass ionomer cements, including ASPA, ChemFil, AquaCem, Fuji I, Fuji II, and Ketac Cem were studied, in addition to MQ Silicate cement. For each cement, five rectangular specimens, 3 x 2 x 0.2 cm were fabricated. Each specimen was suspended in a 20 mL jar of distilled, deionized water at 37°C. Water was changed according to a strict protocol over the course of 30 months. At each change, buffer was added to the water and fluoride content measured in ppm using a fluoride ion electrode. Results showed that all materials had elution patterns with a linear dependence on $t^{1/2}$, which is preceded by a rapid, non-linear diffusion pattern. This, according to the authors, translates to an initial surface phenomenon of fluoride release, followed by a bulk diffusion process which continued until the end of the testing period (about 2 ½ years). In the article, many equations were presented, but none which singularly demonstrated the pattern of fluoride release or diffusion. However, the article does demonstrate that measuring fluoride release patterns from discs or specimens using ion-specific electrodes is certainly dependent on specimen size (surface area). Thus, when comparing different materials for fluoride release, only relative rates of release can be compared—actual amounts of fluoride released become dependent on specimen size. In contrast to ion-specific electrode measurements, by using electron microprobe analysis, a linear profile of fluoride uptake and diffusion into dentin may be determined that is essentially independent of specimen (or restoration) size.

Depending on the material, the amount of fluoride incorporated into the material may or may not have a measurable effect on the amount of fluoride released. For instance, for fluoridated amalgam, research demonstrates that release may be correlated to the amount of fluoride incorporated into the amalgam. However, for glass ionomer and resin-modified glass ionomers, the answer is not as simple. In 2002, Donly and Segura published an article on measuring fluoride release from a resin-modified glass ionomer cement with different levels of fluoride.[23] Also evaluated was the adjacent dentin demineralization. In the study, 25 discs measuring 8 mm in diameter and 2 mm in width were made using P50, a non-fluoridated

composite. Five similar discs were made with either Vitremer, Vitremer loaded with an additional 1 wt. % fluoride (2.2% NaF), 2% fluoride, and 3% fluoride. All specimens were placed in 10 mL of distilled deionized water over 30 days, with daily measurements using a fluoride-specific ion electrode. Each day, the water was replaced with a new 10 mL.

The second phase of the study involved taking 50 extracted mandibular molars and preparing Class V preparations on the mesial surface (6 mm x 4 mm x 1.5 mm). Ten teeth per group were restored with P50, Vitremer, Vitremer 1%, 2%, or 3%. All restorations were finished and polished to ensure no overhangs. Each group of 10 teeth was placed in 500 mL of artificial saliva for 30 days with the saliva replaced every 2 days. The saliva was supersaturated with calcium and phosphate ions. After the 30 days of immersion, the teeth were coated with varnish, leaving 1 mm of sound tooth adjacent to the restorative margins. All teeth were then placed in a pH 4.4 artificial caries solution for 5 days. At the end of the 5 days, the teeth were rinsed and stored in deionized water. Thereafter, 100 μm longitudinal sections were made using a microtome. Tooth margins were photographed under a polarized light microscope, and the demineralized areas examined for the presence of inhibition zones or wall lesions.

Results of the measurements for fluoride release showed that P50 (the non-fluoridated composite resin) released minimal fluoride over the 30 days. For the different Vitremer discs, fluoride release was highest after the first 2 days. The Vitremer specimens with 3% fluoride added had the most fluoride released of any specimens—as the fluoride dose increased, the fluoride release increased.[23] The reduction in fluoride release was about 75-80% from the first day to day 10, and about 35% from day 10 to day 20. For the demineralization phase of the study, the average area of demineralization followed the expected pattern, with P50>Vitremer>Vitremer 1%>Vitremer 2%>Vitremer 3%. The results of this experiment are significant in that they demonstrate a dose-response relationship for glass ionomers-based materials. From this study, it appears that the higher the amount of fluoride within the material, the higher the fluoride released. Also, with glass ionomer based material having documented recharging capabilities, periodic use of topical fluorides may lead to continued significant fluoride release. One limitation to this study is that it only demonstrates a dose-release relationship with a single material that has been loaded with different fluoride doses. The applicability of this research toward comparing different commercial glass ionomer and RMGIC

products based on their fluoride content is still questionable, as composition, glass particle size, and resin filler components are often different.

Direct measurement of fluoride uptake into tooth

As demonstrated above, the use of techniques such as fluoride ion-specific electrodes to measure fluoride release can be valuable. Perhaps even more significant is research that directly measures fluoride uptake into dentin from restorations. In 1985, Weatherell et al. published an article describing a microsampling technique with chemical analysis. With the technique, it was possible to analyze microscopically thin layers of dentin under restorations. Using the described technique, Mukai et al. (1993) measured the fluoride uptake in dentin from glass ionomer cements.[24] In the study, nine 6th grade students were selected and had the two preparations placed in the occlusal surface of their maxillary third molars, each with dimensions of 2.5 mm deep and 1.5 mm in diameter. For each tooth, two restorations were placed, with Vitrabond (a F-releasing glass ionomer), and zinc phosphate (Elite cement 100, with 0.74 wt. % F). The teeth were left intraorally for 3 months and then extracted. Three longitudinal cuts were made through each of the two restorations using a diamond disc, and polished to about 400 μm thickness. From each of the tooth sections, four specimens measuring about 3 mm in length, 400 μm thick, and 700 μm in width were made. Altogether, 36 adjacent specimens were obtained, measuring 700 μm wide. These specimens were attached to rods and abraded in layers of about 30 μm using Imperial Lapping Film (3M). The powder from each abrasion procedure was dissolved in a solution. Phosphorus concentration was determined with chemical analysis and the fluoride concentration was determined using a modified F electrode. A ratio of F:P was established assuming average P concentrations in dentin.

Results of the experiment showed that fluoride concentrations were highest directly under the restorative materials (about 1 mm from the dentino-enamel junction) and decreased toward the pulp, increasing again when nearing the pulpal surface. The average penetration of fluoride into dentin was approximately three-fifths the distance from the restoration to the pulp, occluso-pulpally; at about three-fifths of the distance from the restoration-dentin surface to the pulp, the fluoride levels for the Vitrabond-restored specimens decreased to the levels of unrestored dentin. The total, average, and maximum fluoride concentrations were significantly higher with teeth

restored with Vitrabond compared to zinc phosphate. The maximum fluoride concentration for teeth restored with Vitrabond and zinc phosphate was about 7,000 ppm and 1,000 ppm, respectively. The *average* fluoride concentration in dentin was about 2,400 ppm for Vitrabond, and near zero for zinc phosphate. These results indicate that fluoride within dentin may penetrate quite deep. One drawback of this method of analysis, however, is that increments of 30 μm were used for fluoride analysis. Furthermore, there was only a single time interval (3 months) between restoration placement and fluoride analysis.

A more direct method of fluoride measurement within tooth structure can be accomplished using electron microprobe analysis. Since the proposed research involves such analysis, it is important to review any important articles pertaining to the distinctive method of analysis. A review of X-ray microanalysis by Hals et al. (1988) produced many salient points that are applicable to the proposed research study.[25] In particular, Hals et al. pointed out that treating a cavity wall with 2% NaF solution prior to filling results in a small increase of fluoride within the dentin walls. In some studies, no increase in fluoride concentration could be detected when a fluoride-containing liner was used.[26] It has been reported that the addition of fluoride to filling materials such as amalgam will increase fluoride within dentin. Indeed, in many studies, fluoride concentrations have been increased within dentin after exposure to fluoride-containing amalgam. All these points are well-documented by electron microprobe analyses as reported in many of the following articles.

Although the point is debatable, according to Figures et al. (1990), measurement of fluoride uptake into enamel by electron microprobe analysis is as accurate as measurement by chemical methods.[27] In the study by Figures et al., canines from patients over 50 years of age were extracted, sectioned horizontally at the CEJ, mounted, polished, and immersed in NaF neutral solution of either 2, 1.5, 1, 0.5, and 0.05% concentrations for 7 days at 25°C, stirring daily.[27] Subsequently, the teeth were sectioned longitudinally through the root canals and dried for 2 weeks. Using a microprobe with a probe size of 1 μm, three parallel linear analyses for fluorine were made from the root face down toward the root apex for each specimen. At each analysis position, fluorine levels were measured both over a tubule (peritubular dentin) and over adjacent dentin (intertubular dentin).[27] Results showed that 2% NaF (9,000 ppm F ion) immersion resulted in significant fluorine uptake up to 400 μm in peritubular dentin, but only up to 100 μm in intertubular dentin. With 1.5% solution, uptake was to a depth of 200 μm and 40

μm for peritubular and intertubular dentin. For 1.0% solution, uptake was up to 60 μm. At lower NaF concentrations, the specimens showed minimal detectable uptake of fluorine. No exact numbers in parts per million of fluorine taken up in dentin were reported. These results demonstrated that there were differences between intertubular dentin and peritubular dentin regarding fluorine uptake. With higher surface area, fluoride levels were consistently higher in peritubular dentin compared to intertubular dentin. Only at high concentrations of fluoride exposure did the dentin take up fluoride. In a thorough literature search, this is the only study found that aimed to measure differences in fluorine uptake between intertubular and peritubular dentin. All other studies have analyzed dentin as though it were a homogeneous material.

Prior to the development of glass ionomer and composite materials, amalgam was the most commonly used direct restoration material in dentistry. Consequently, the first electron microprobe studies of fluoride release from restorations involved fluoridated amalgam. Although fluoride-containing amalgam is less common currently, in part due to reduced mechanical properties, many studies have demonstrated significant fluoride release from such amalgams. In a study by Skartveit et al. (1990), the in vivo fluoride uptake in enamel and dentin was studied.[12] Using children aged 8-9, restorations in primary molars were placed using either fluoride-containing amalgam (FluorAlloy) with 1% stannous fluoride or glass ionomer cement (Fuji II F) with 11.3% fluoride concentration in the powder. One to two years after restoring, the primary teeth were exfoliated. Six teeth were available for examination—two with a FluorAlloy restoration, four with Fuji II F. Also, three primary molars with New True Dentalloy fluoride-free amalgam were used as controls. Finally, four adult premolars scheduled for extraction for orthodontic reasons were sealed with glass ionomer sealant (Fuji III) and the contralaterals sealed with Concise, a fluoride-free resin. After two weeks, the teeth were extracted and stored in water. Microprobe analysis of 200 μm thick sections was performed. Linear analyses for F, Si and Ca were made at right angles to the cavity and fissure walls. The Ca analysis was used to determine the position of the tooth-restorative interface. Concentrations of fluoride were estimated using a fluorapatite standard to create a linear graph. Results analyzing fluoride concentration showed that fluoride in the glass ionomer (Fuji II F) group ranged from 1.2 to 3.8% in dentin, and from 0.2-2.9% in enamel. For the FluorAlloy group, fluoride ranged from 0.6-0.9% in dentin. For the Fuji III (fluoride sealant) group, fluoride concentrations ranged from 0.2-1.9%. Penetration of fluoride from glass ionomer ranged from 220-750 μm in dentin and

180-400 μm in enamel. Penetration of fluoride from FluorAlloy reached depths of 110-550 μm in dentin, while Fuji III sealants demonstrated fluoride penetration of 40-260 μm in enamel. Unfortunately, the clinical applicability for the fluoride-containing glass ionomer sealant, Fuji III, may be undermined as the most accepted standard for sealants currently is to use unfilled or low-filled resin composites.

In another study by Tveit and Tötdal (1981), the fluoride uptake from a fluoride-containing amalgam was measured.[28] Ten premolars extracted for orthodontic reasons were prepared with Class V cavities and filled with FluorAlloy fluoride-containing amalgam or New True Dentalloy conventional amalgam. All teeth were then stored in 37°C artificial saliva (pH 5.2) for 3 months, with the solution changed weekly. At the end of the 3 months, each specimen was sectioned into 3 or 4 bucco-lingual slices for electron microprobe testing. Microprobe analysis was done, measuring for F, Ca and P simultaneously. Analysis speed ranged from 20-146 μm/min along the tooth-restorative interface. In order to estimate approximate fluoride concentrations, a fluorapatite mineral was used as a standard. A linear relation between concentration and analysis intensities was developed from the standard. Results showed that there were no fluoride concentrations above the 1,500 ppm detection limit in the specimens restored with conventional amalgam. In the FluorAlloy specimens, the fluoride-rich layer in enamel and dentin was 27 ± 27 and 108 ± 54 μm, respectively. The F concentrations in dentin were above detection limits for all FluorAlloy specimens, with 80% of analyses ranging from 0.9-1.5%. A maximum of 1.9% F concentration was reached. The results from this study were within the ranges seen in other studies measuring fluoride uptake from fluoride-containing amalgam. As seen with most other studies, the fluoride uptake in dentin was significantly higher than that in enamel, due to the higher porosity and reduced mineralization of dentin.

A research article by Sougandis et al. (1981) demonstrated the use of electron microprobe analysis to measure fluoride release by amalgams containing fluoride.[29] In the study, caries-free premolars scheduled for extraction for orthodontic reasons were prepared with labial Class V cavities and restored with either regular cut, or fine-cut amalgams containing either 1% or 5% SnF_2. Four groups of ten teeth each were restored with either regular 1%, regular 5%, fine-cut 1%, or fine-cut 5% SnF_2 amalgam. These groups were then divided in two, and half the teeth were extracted 15 days after restoring, half the teeth were extracted 30 days after restoring. After extraction, teeth were dried, mounted, and sectioned bucco-lingually across the amalgam/tooth

interface. Without polishing, examinations were then carried out using a stepwise line analysis at 10 μm intervals. Calibration using a hydroxyapatite standard was done to determine that 10 counts corresponded to about 75 ppm of F ion. Measurements were made from the tooth-restorative interface inward along the DEJ. Results show that there was a significant uptake of fluoride by dentin and enamel, with penetration ranging from 10-100 μm. Fluoride reached peak levels of over 1,500 ppm near the tooth-amalgam interface for 5% SnF_2 fine-cut amalgam after 15 days, which was the maximum. Penetration was higher for fine-cut amalgam compared to regular amalgam. Also, the group with 5% SnF_2 had significantly more fluoride uptake than the 1% group. Finally, at 15 days, for every group, fluoride uptake was higher than after 30 days. Similar results have been reported by others. The reasoning behind this finding was that fluoride may diffuse into saliva from the dentin, or slowly toward the pulp, thus decreasing concentration. Also, as expected, fluoride release may be rapidly decreased from the amalgam. Such mechanisms may also occur with other materials, such as glass ionomer cements or resin-modified glass ionomers.

While most current amalgam compositions do not include fluoride, there are other methods to apply fluoride to a cavity preparation when restoring with amalgam. For example, Tveit (1980) measured fluoride uptake by cavity walls following the application of Duraphat fluoride varnish around amalgam restorations.[30] Using 11 premolars extracted for orthodontic reasons, Class V and I cavities were prepared and restored with or without Copalite, followed by amalgam. Finally, Duraphat, containing 50 mg/mL NaF was applied after immersion in water for 5 minutes. Teeth were stored another 12-24 hours, then sectioned into 200-300 μm thick sections. Another six teeth were prepared in vivo, half lined with Copalite, and restored with amalgam. Duraphat was applied, and the teeth were extracted a week later. Electron microprobe analysis of the in vitro and in vivo specimens was done. These analyses demonstrated a 10-20 μm wide layer of increased fluorine concentration in enamel, as well as a narrow 10-30 μm inner layer in dentin. Most of the specimens both in vivo and in vitro demonstrated significant fluoride increases within dentin when not lined with Copalite prior to restoring with amalgam. The concentrations of fluoride ranged from 2,000-3,500 ppm in the in vitro specimens and from 2,000-6,000 ppm in the in vivo specimens. In this study, fluoride uptake was measured from every area along the cavity walls, and demonstrated that fluoride uptake and penetration into dentin was much greater than into enamel. With specimens varnished with Copalite prior to

restoring, only a few teeth demonstrated increased fluoride concentrations. It was postulated that the Copalite diminished the effect of the Duraphat by creating a barrier for fluoride penetration. In the few instances that fluoride did penetrate into dentin, the authors presumed microleakage occurred.

In a study of fluoride uptake from various different types of materials, Tveit et al. (1987) used electron microprobe analysis to measure differences between a fluoride solution, a liner, and a fluoride-containing amalgam.[26] The authors suggested that these three materials may be placed into cavity preparations prior to restoring with amalgam. Using three adult beagles, maxillary first molars and canines were drilled with Class V preparations on the buccal surfaces, with the depth extending about 1 mm into dentin. Walls were finished with finishing burs, and the left teeth restored with New True Dentalloy amalgam (control group). The right side restorations were either pretreated with 2% aqueous NaF for two minutes and restored, lined with Tubulitec (Dental Therapeutics AB) and restored, or restored with FluorAlloy with 1% SnF_2 without any pretreatment. Five months later, the dogs were killed and each tooth cut in 3-4 longitudinal sections about 200 μm in thickness. The amalgam restorations were all lost in this procedure. Sections were cleaned, polished and analyzed with an electron microprobe operating at 10 kV and 50 nA. Linear analyses were made at right angles to the cavity walls, only in dentin. Six analyses were made per cavity. Element concentrations were estimated, assuming a linear relation between concentration and X-ray emission intensity compared to those of a fluorapatite standard (correcting for background).[26] The results of the study showed that those preparations treated with NaF showed fluoride increases exceeding the 0.15% detection limit in about 2/3 of the analyses, with a maximum concentration of 0.6%. Mean penetration depth was only 13 μm. The cavities treated with a liner, fluoride concentrations didn't exceed the detection limit. For walls exposed to FluorAlloy for 5 months, the average fluoride concentration was 0.53%, with a maximum of 0.88%. Average penetration was 62 μm.

This study demonstrated that an electron microprobe can be used successfully to show the fluoride content in dentin at the cavity walls. The results demonstrated that fluoride applications such as 2% NaF under amalgam may result in minimal fluoride incorporation into dentin. Also, the research seemed to discount the release of fluoride from liners such as Tubulitec, as fluoride concentrations were undetectable under such liners. However, the study showed results with fluoride-containing amalgams that were similar to other studies.

Electron microprobe analysis of glass ionomer and hybrid ionomer fluoride release

With greater implications than fluoride release from fluoridated amalgam, some studies have used electron microprobes to measure fluoride uptake in dentin from glass ionomer-based materials. In 1980, Wesenberg and Hals published a study on glass ionomer restorations in Class V cavity preparations using electron probe microanalysis.[31] In their study, six unconditioned Class V cavities were prepared at the CEJs of extracted teeth and filled with ASPA glass ionomer cement. The ASPA cement was reported to have an 11.5% concentration of fluoride in the pre-reacted powder. Restored teeth were stored for 1-3 months in distilled water at 37°C and subsequently sectioned bucco-lingually and studied by microradiography. Some of the sections were also analyzed using an electron microprobe. Analyses for Ca, P, Mg, Na, F, Al, and Si were performed with a 10 kV pulse height at right angles to the sections, through enamel and dentin. Microradiography results showed that there was an increased radiopaque area about 20 μm thick into dentin from the restorative-tooth interface. It was hypothesized that these areas were due to increased Ca and P. With a low initial pH of 2.5, the glass ionomer may cause a superficial demineralization. With fluoride uptake into the dentin, Ca, P and Mg ions may be precipitated from the demineralized hydroxyapatite into new calcium salts. The glass ionomer may also be a source for Ca and P ions. Electron probe results showed increased concentrations of F and Al in enamel and dentin of 0.3% and 1.5%, respectively. These zones decreased to <0.1% at a distance of 6-80 μm for F and 8-24 μm for Al from the surface. Thus, it was shown that fluoride penetrates to a lesser extent into enamel than dentin under these conditions. Furthermore, it was demonstrated that fluoride uptake does occur in dentin and can be measured using an electron microprobe.

In research utilizing not only a fluoride ion-selective electrode, but an electron microprobe for fluoride analysis, Han et al. (2001) studied the effect of a fluoridated adhesive resin cement on tooth structures.[32] Using Clearfil Photobond as a control, Fuji liner LC (glass ionomer liner), Fluorocement (fluoridated adhesive resin cement), and Teethmate F (fluoridated sealant) were tested for fluoride release into solution. Specimens with 9 mm diameter and 1 mm height were created from each material and kept in deionized water at 37°C. Parts per million fluoride ion were measured using a fluoride ion-selective electrode after 1, 7, 30, and 60 days. Five specimens were prepared for each test material. Solutions of NaF with 0.1, 1, 10, and 100

ppm were made to create a standardization graph using a fluoride ion-selective electrode. Also, the authors measured fluoride uptake by tooth, using premolars extracted for orthodontic reasons. Class V preparations were made to a depth of about 2 mm, conditioned according to manufacturer's instructions, and restored and light-cured with the various materials. Margins were finished with a fine diamond bur. Individual specimens were kept in 20 mL of deionized water at 37°C for 60 days. At that time, they were sectioned through the middle with a microcutter and analyzed using a WDX electron probe microanalyzer.

Results of the fluoride release experiment showed that the Fluorocement released the most fluoride after 7 days, followed by Fuji liner LC, Teethmate F, and the control. Most of the fluoride was released after the first day, with minimal amounts after 7, 30, and 60 days. However, equilibration between the solution and restorative may account for some of the reduction in fluoride release observed after the first day immersed. Results of the electron microprobe analysis showed that Fluorocement released fluoride after 60 days and concentration in dentin was higher than in enamel. After just 1 and 7 days, minimal fluoride was released into dentin. The results of the study indicated to the authors that fluoride release and uptake by tooth structures was higher with the fluoride-containing adhesive resin cement (Fluorocement) than by glass ionomer liner (Fuji liner LC) or the fluoride-containing fissure sealant (Teethmate F).[32] The increased fluoride uptake into dentin compared to enamel was believed to be due to 1) dentin tubules, 2) the presence of collagen fibers, 3) smaller size of apatite crystals, and 4) lower mineral content and higher water content.[32] Another postulation by the authors was that small, microscopic gaps between the cavity walls and the restorations may cause microleakage, leading to greater fluoride release and uptake by dentin. The finding that a fluoridated resin cement (Fluorocement) released more fluoride than a glass ionomer based liner (Fuji liner LC) is very interesting. This finding is similar to that of Tveit et al. (1987). The question remains however, whether the fluoride was released because of microleakage at the Fluorocement resin-dentin interface. This question may have been answered by Ferracane et al. (1998).

In an article reporting on the fluoride release from a dentin adhesive, Ferracane et al. (1998) evaluated microleakage and fluoride levels at the tooth-restorative interface of Class V restorations.[33] Also, studied was the fluoride release from fluoride-containing adhesive resin discs. Two dentin adhesives were evaluated for fluoride release: 1) FB, a 3-component system with 7% phosphoric acid etchant, a two-part primer, and a light-cured adhesive with 10 vol %

fluoride-containing filler, and 2) Scotchbond MP (SBMP), a 3-component system with 35% acid etchant, a primer, and light-cured adhesive, with no filler. Z100 composite was used with the Scotchbond MP, and Litefil used with FB. Specimens measuring 4 mm in diameter and 2 mm in thickness were made with FB adhesive for testing fluoride release. The specimens were placed in deionized water at 27ºC for 1-112 days. Solution was changed at each measuring period and fluoride analysis was done using a fluoride specific ion electrode. Results of the fluoride release from FB specimens in solution showed an initial release rate of 0.096 ppm/day, declining to 0.02 ppm/day at steady state.

Additionally, microleakage from Class V cavities in third molars was examined. Using both the buccal and lingual surfaces, Class V preparations were made and restored with either FB and Litefil, or SBMP and Z100. Restorations were finished with Sof-lex discs and stored in deionized water at 37ºC for 1 month. Subsequently, specimens were placed in silver nitrate for 24 hours, washed, and immersed in developing solution for 24 hours. Specimens were then mounted, sectioned through the middle of the restorations, and evaluated for microleakage using a microscope. Fluoride measurement of these specimens was done using an electron microprobe. Analyses were done at 10 kV and 10 nA, going from the bonding resin through the hybrid layer into the dentin. A linear analysis 16 μm long with points measured every 0.5 μm were made for each specimen in selected leakage areas, as well as non-leaking areas. To image the hybrid layer edges, P and Ca were also measured along with fluorine. Results indicated that there was no difference in leakage between FB and SBMP at either enamel or gingival margins. With the electron microprobe analysis, peak counts for fluorine were only above background level in areas where microleakage occurred. The average of these counts (minus background) in dentin below the hybrid layer was 90 counts, translating to about 3,800 ppm F for FB/Litefil restored specimens. The elevated fluorine content appears to be only in the first 10 μm of dentin beyond the hybrid layer, with the most elevated content at the hybrid layer base (contacting dentin directly). The conclusions from the research were:

1) Neither adhesive was entirely successful in eliminating leakage in Class V restorations.
2) No restorations demonstrated leakage up to the pulpal wall.
3) The leakage areas demonstrated that fluoride could be released from gaps less than 10 μm and taken up by dentin.

4) Fluoride above detection limits was not identified in dentin below adhesive when leakage was not evident. Thus, for fluoride to be released from the FB adhesive, water penetration was necessary for transport.

A series of articles by Yamamoto and colleagues are a guideline for the primary research approach of the proposed research project. In an in-depth electron microprobe analysis of fluoride uptake, Yamamoto et al. (2000) investigated fluorine levels around cavities restored with various materials.[6] The difference between this research and other electron microprobe studies is that the authors investigated the entire cavity preparation for fluoride distribution. Two separate experiments were conducted by Yamamoto et al. The first part of the research involved NaF application to Class V cavity preparations. Extracted premolars were used and two cavities drilled in each buccal surface. In half of the preparations, 2% NaF solution was applied for four minutes. The other half was used as controls. Each tooth was then immersed in saline solution at 37°C, with NaF application repeated every 3 days and saline solution renewed. Following 12 days (4 applications of NaF), the teeth were immersed in saline without further NaF exposure, and the solution was renewed weekly. One month thereafter, the teeth were mounted, sectioned through the axial walls, and polished to 0.05 μm. The effect of damage by cutting and polishing was checked by the researchers and determined to have no observable effect on the specimens.[6] Fluoride distribution around the cavity walls was measured using WDX, with analysis points taken every 300 μm from the line angle of the preparation. Settings included an acceleration voltage of 15 kV, a probe current of 0.05 μA, a beam focus of 1 μm x 5 μm, and a travel speed of 10 μm/min. A calibration curve was made from standard samples with 0, 25, 50, 75, and 100% fluoride exchanged for hydroxyl groups in hydroxyapatite. From these standards, ppm of fluoride could be estimated, as a linear regression was developed.

The results of the fluoride mapping of the teeth with NaF applications were that fluoride uptake was higher generally in dentin than in enamel, but less location-dependent in enamel than in dentin.[6] Surface fluoride concentrations in dentin were between 5,500-9,700 ppm at the side walls of the preparations and between 5,700-11,000 ppm at the axial walls. The concentration decreased quickly perpendicular to the cavity walls, though the rate of decrease was dependent on location. For instance, the decrease occurred much faster at the occlusoaxial line angle. In this region, penetration of fluoride was very shallow, reaching only about 20 μm into the tooth.

In dentin, average penetration of fluoride was 125 μm at the side walls (ranging from 48-215 μm), and was 138 μm at the axial wall (ranging from 66-219 μm).
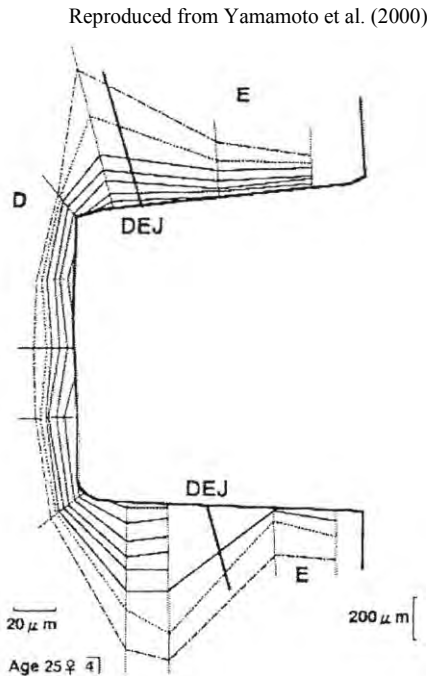
Figure 1. Representative mapping of the fluoride distribution around a cavity wall of a tooth filled with Fuji II. Each line represents 1,000 ppm, with the outermost lines representing 500 ppm and the detection limit.[6]
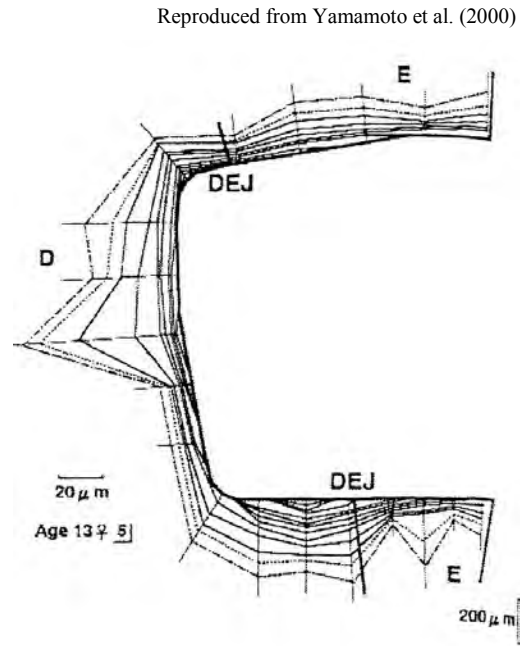
Figure 2. Representative mapping of the fluoride distribution around a cavity wall of a tooth filled with Fuji II LC. Each line represents 1,000 ppm, with the outermost lines representing 500 ppm and the detection limit.[6]

The second part of the research by Yamamoto et al. was to map fluoride within teeth restored with various fluoride-releasing materials. Preparing Class V cavities similar to those described above, the teeth were filled with either Fuji II (glass ionomer), Fuji II LC (RMGIC), Teethmate F (fluoridated sealant), or Kurasper F-F-bond and Silux Plus (bonding agent and non-fluoridated composite) as recommended by the manufacturers. The Teethmate F and Silux Plus cavities were etched for 40 seconds prior to restoring. Fuji II and Fuji II LC restorations were placed, and varnished with GC Fuji varnish to protect the surface. Three specimens per restorative material were fabricated, and immersed in 10 mL saline at 37°C for one month, with the solution renewed weekly. The specimens were then sectioned, polished, and analyzed using an electron microprobe as before. The results of the analyses are shown in Figures 1, 2, 3, and 4.

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At side walls in dentin, fluoride concentrations averaged about 5,700 ppm, 6,700 ppm, 6,300 ppm, 11,800 ppm, for Kurasper F-F Bond and Silux plus, Fuji II, Fuji II LC, and Teethmate F, respectively. At the axial wall, fluoride concentrations of about 4,900 ppm, 5,700 ppm, 5,600 ppm, and 8,900 ppm were recorded for the respective materials. Average penetration depth at side walls was about 16 μm, 44 μm, 45 μm, and 86 μm for Kurasper and Silux, Fuji II, Fuji II LC, and Teethmate F, respectively. At the axial walls, average fluoride penetration was about 16 μm, 23 μm, 38 μm, and 88 μm for the various materials.
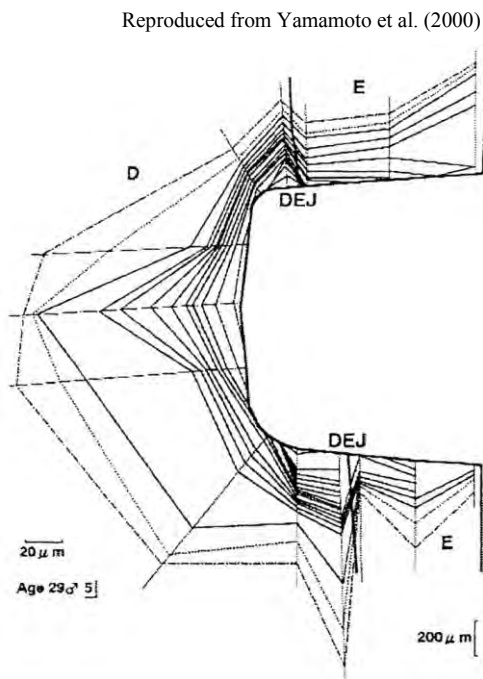
Figure 3. Representative mapping of the fluoride distribution around a cavity wall of a tooth filled with Teethmate F. Each line represents 1,000 ppm, with the outermost lines representing 500 ppm and the detection limit.[6]
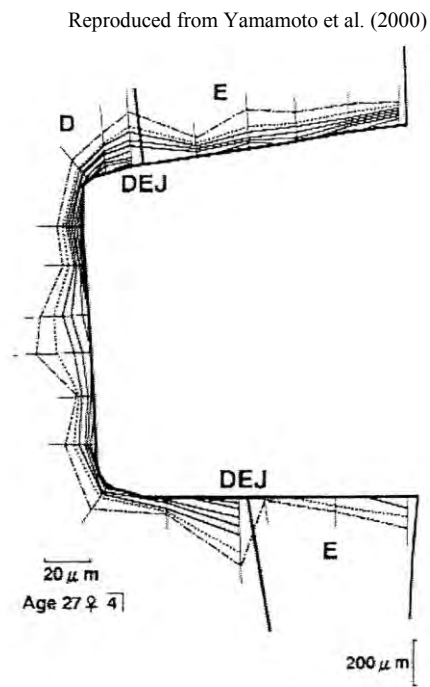
Figure 4. Representative mapping of the fluoride distribution around a cavity wall of a tooth filled with Kurasper F-F-bond followed by Silux plus composite. Each line represents 1,000 ppm, with the outermost lines representing 500 ppm and the detection limit.[6]

From the results of the research, the authors concluded that there is a strong location dependence of the fluoride uptake in a tooth.[6] Partly, this may be because dentinal tubules run relatively perpendicular to the axial wall of Class V restorations, while at the occlusoaxial line angle, for instance, the tubules run fairly parallel. Thus, at the axial wall, with open tubules,

fluoride may penetrate faster and further than other regions of a preparation. A very important finding by Yamamoto et al. was that the fluoride uptake of NaF, Teethmate F, Fuji II, and Fuji II LC did not appear to coincide with the relative amount of fluoride released into saline, as measured in a previous experiment.[6] Thus, it appears as though the 2-D mapping generated through electron microprobe analysis is important in evaluating true amounts of fluoride uptake into dentin. The abbreviated conclusions of the article were as follows:

1) The amount of fluoride uptake depended on location.
2) Fluoride uptake in dentin was higher than in enamel.
3) Fluoride uptake from fluoridated resin (Teethmate F) was more than from a glass ionomer restorative (Fuji II).

The exact reason why there was greater fluoride uptake around Teethmate F restorations compared to the glass ionomer restorations was not determined. One possible explanation may be because Teethmate F is a low-viscosity resin, thus penetrating into dentinal tubules when applied, forming resin tags and allowing a deeper initial penetration of the material. Another reason may be because the Fuji II and Fuji II LC specimens were covered with a protective varnish after restoring, possibly prohibiting external moisture from leaking in. Moisture and microleakage may be very important factors in fluoride release from restoratives. Therefore, there is a question of whether in vitro versus in vivo techniques would produce different results.

To answer this question, Yamamoto et al. (2001) recently published a study to determine any differences between in vivo and in vitro conditions on the fluoride uptake from Teethmate F-1 light-cured composite resin with fluoride.[34] The goal of this study was to determine the uptake of fluoride around a tooth cavity wall from a fluoride-releasing material in vivo using microprobe analysis, and compare the results to in vitro data. As described above, Yamamoto et al. (2000) studied two-dimensional mapping in vitro. Under in vivo conditions, dentin tubules are supposedly filled with dentinal fluid, are hydrodynamic, and may affect fluoride uptake.

For in vivo specimens, Class V cavity preparations were done on the buccal surfaces of maxillary and mandibular premolars, etched, and filled with Teethmate F-1 composite resin and cured for 40 seconds. After 1 month, the teeth were extracted carefully, bisected through the axial wall, polished, and analyzed with a WDX microprobe. In vitro specimens were treated

similarly, except they were coated with nail polish after restoring, immersed in 10 mL of saline
and kept at 37°C for 1 month, renewing the saline every 1 week.
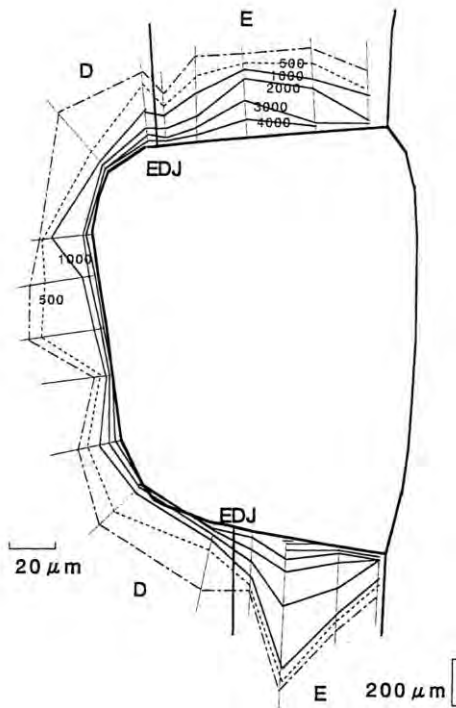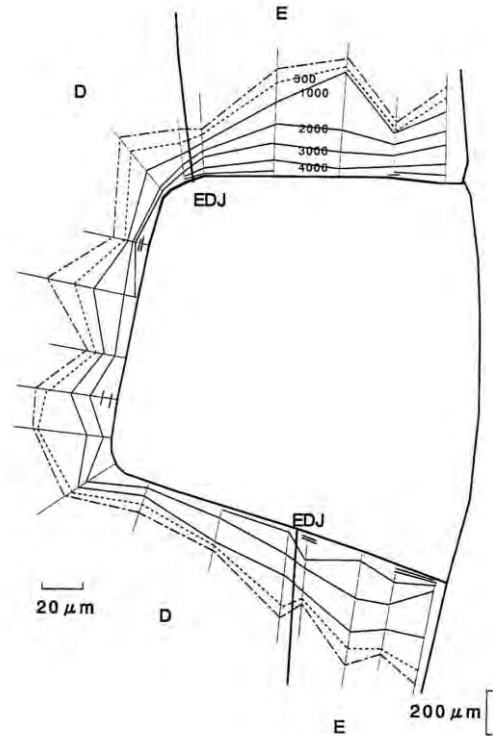
Figure 5. Fluoride distribution around the
cavity wall of a tooth filled with Teethmate
F-1 in vivo. The lines represent 4,000, 3,000,
2,000, 1,000, 500 and baseline ppm F.[34]

Figure 6. Fluoride distribution around the
cavity wall of a tooth filled with Teethmate
F-1 in vitro. Lines represent equivalent ppm
of fluoride.[34]

Measuring fluoride in the teeth, linear analyses were taken going around the cavity walls
every 300 μm from the line angles. Fifteen kV and 0.05 μA were used for X-ray production,
with a speed of 10 μm/min travel speed. Using a partially fluoridated hydroxyapatite, a
calibration curve of fluoride concentration versus WDX intensity was constructed.[34] Using
standards of 0, 25, 50, 75, and 100% fluorapatite in hydroxyapatite, a standard line graph was
created. Figures 5 and 6 demonstrate two maps of fluoride distribution for in vivo and in vitro
specimens. The overall results of the microprobe analyses showed no statistical differences
between in vivo and in vitro teeth in regards to fluoride uptake. Maximum fluoride uptake in
dentin reached 6,900 ± 1,600 ppm in vivo, and 6,700 ± 1,600 ppm in vitro. Maximum fluoride

penetration depths were $40 \pm 17 \mu m$ for in vivo, and $79 \pm 40 \mu m$ for in vitro specimens. This study appears to disprove the hypothesis that in vivo specimens will have different levels of fluorine uptake than in vitro specimens, due to intertubular fluids and pressures. With the fluoride mapping, similar uptake was found between in vivo and in vitro specimens when restored with fluoride-containing Teethmate F-1.

In an earlier article, Yamamoto et al. (1996) described the time-dependent change of fluoride uptake around cavity walls.[35] Only the abstract was available in English. The research methodology applied WDX (wavelength dispersive X-ray) analysis to measure fluoride uptake in dentin at one, three, and six month immersion periods when in contact with a conventional glass ionomer restoration. Results reported were that: 1) fluoride uptake varied depending on the specific place measured, 2) fluoride penetration increased with time at the axial wall of the restoration, and 3) surface fluoride concentration was not different between the three different immersion durations. While the principles of this study are very similar to the proposed research, differences are evident. For instance, one, three, and six month immersion periods were used in the research. Additionally, a conventional glass ionomer material was studied, while in the proposed research, a resin-modified glass ionomer will be used.

Fluoride inhibition of demineralization

Fluoride release from a restoration is inconsequential if it doesn't result in an inhibition of demineralization. Therefore, it is important to identify studies that determine the ability of restorative materials to prevent decay. For instance, the previously mentioned study by Donly and Segura (2002) is very significant in that different fluoride concentrations (within the restorative material) were related to the inhibition of demineralization, as measured by polarized light microscopy.[23] Measuring zones of demineralization inhibition is an indirect method of assessing fluoride uptake from restorative into tooth structure. Larger (or wider) zones of inhibition translate to deeper penetration of fluoride into the dentin. It also may indicate a larger amount of fluoride was released from the restorative. A study by Pereira et al. (1998) investigated the inhibition of demineralization around fluoride-releasing materials.[36] Fuji II LC (RMGIC), Vitremer (RMGIC), Fuji II (GIC), and an adhesive system (Clearfil Liner Bond II, Protect liner F, and Clearfil AP-X) were used to fill either buccal or lingual Class V preparations

on bovine incisor roots. All specimens were stored in tap water for 1 week at 37°C, then finished and polished flat. Each specimen was then stored in 20 mL of buffered demineralizing solution (pH 4.5) for 3 days at 37°C. After the demineralizing step, sections about 150 μm thick were cut through each restoration, perpendicular to the long axis of the root, and polished to about a 100 μm thickness. Specimens were then dehydrated and observed under a polarized light microscope. Results showed the widths (from restorative into tooth structure) of the inhibition zones adjacent to Fuji II were significantly larger than those adjacent to Fuji II LC and Vitremer. The heights of the zones (from tooth surface inward) were similar for Fuji II and Vitremer, but were significantly higher than the zone around Fuji II LC. No zone of inhibition was observed for the Clearfil AP-X composite resin specimens. These results indicate that Fuji II (a pure glass ionomer) had the largest zone of inhibition (width and height), compared to Fuji II LC and Vitremer, both RMGICs. Even though Clearfil Liner bond II reportedly has fluoride, the amount released may have been insufficient create a zone of inhibition.

In another interesting article by Tsanidis and Koulourides (1992), an abrasion biopsy method was used to determine the fluoride uptake from glass ionomer cements in vitro.[2] Additionally, inhibition of demineralization was investigated. In their research, bovine dentin slabs were examined 1, 7, 14, and 30 days after restoring with glass ionomer cements. Tooth slabs measuring 3 x 5 x 2 mm were cut with a diamond saw under water spray and polished flat down to about 1 mm thickness using 30 μm grit lapping film. The authors reported that all the different slabs had fluoride content consistently less than 200 ppm. Using Fuji II and Ketac-Fil, the slabs were restored with 3 x 5 x 3.2 mm masses of either restorative material. Restorative surfaces other than the dentin-restorative interface were then covered with varnish supplied by the manufacturers. After restoring, the specimens were suspended in synthetic saliva at pH 7.0, with the solution changed every 2 days. The authors reported that keeping the dentin unsealed could simulate tissue hydration as found in vivo. Next, abrasion biopsy was used. The glass ionomer restorations were removed from the dentin specimens using finger pressure, and specimens assessed in 10 μm increments for fluoride concentration. Abrasion was done using 15 μm grip lapping film with the dentin mounted in composite so the abraded surface would be evenly abraded. Solutions were collected along with the cut strips for abrasion, vibrated for two seconds and left overnight for complete dissolution of the dentin. Finally, the strips were

removed and solutions analyzed using a fluoride ion-specific electrode. Abrasion depths were measured to an accuracy of 1 μm using a micrometer.

In addition to fluoride measurements, an acid resistance test was performed using six dentin slabs exposed to the glass ionomers for 6 days, then to acid buffer for 4 days.[2] Initially, each slab was covered two-thirds with varnish, with one-third exposed to the glass ionomer. Then, the varnish was removed and placed only on the middle third of the slabs (where the glass ionomer was), and the slabs were exposed to the acid buffer. Therefore, according to the authors, one-third of each slab was exposed to the glass ionomer and then the acid, one-third was protected from the glass ionomer but then exposed to the acid, and one-third was protected from both glass ionomer and acid exposure.

Microradiography results of the acid resistance test showed a radiopaque zone in areas exposed to the glass ionomer for six days (ARZ—acid resistant zone), while unexposed controls did not have such a zone. There were no differences between the glass ionomers (Fuji II or Ketac-Fil). The ARZ widths were about 40 μm. Results of the other research showed that after 1, 7, 14, and 30 days of glass ionomer exposure, fluoride penetration reached at least 50 μm for all specimens, with the highest fluoride levels at the first layer—10 μm into the tooth. In fact, fluoride concentration dropped off dramatically for 1, 7, and 14 day specimens between the first and second layers (from 10 μm to 20 μm from the interface).

There were statistical differences between the one day slabs and the 7, 14, and 30 day slabs, although not for each 10 μm layer increment. From day 1 to 30, the first layer (abraded from 0-10 μm into dentin) had a 2-fold increase in fluoride concentration, and the fifth layer (40-50 μm into the tooth) had a 3-fold increase. Slabs of Ketac-Fil had higher values of fluoride concentration than Fuji II slabs, but the differences were not significant. Figure 7 demonstrates the decreasing fluoride concentrations from the Ketac-Fil/dentin interface. There are some limitations to this research. Among them is the fact that bovine dentin was used. Furthermore, abrasion biopsy depths are approximate. Finally, the authors indicated that glass ionomer material may have remained attached to the bovine specimens when testing for fluorine content, therefore contaminating the surface (layer 1) and altering the data. Nonetheless, this research is very similar in concept to electron microprobe analysis, evaluating dentin in steps for fluorine content, as well as measuring the fluorine uptake over time.

Fluoride concentration
(mg F/cm^3)
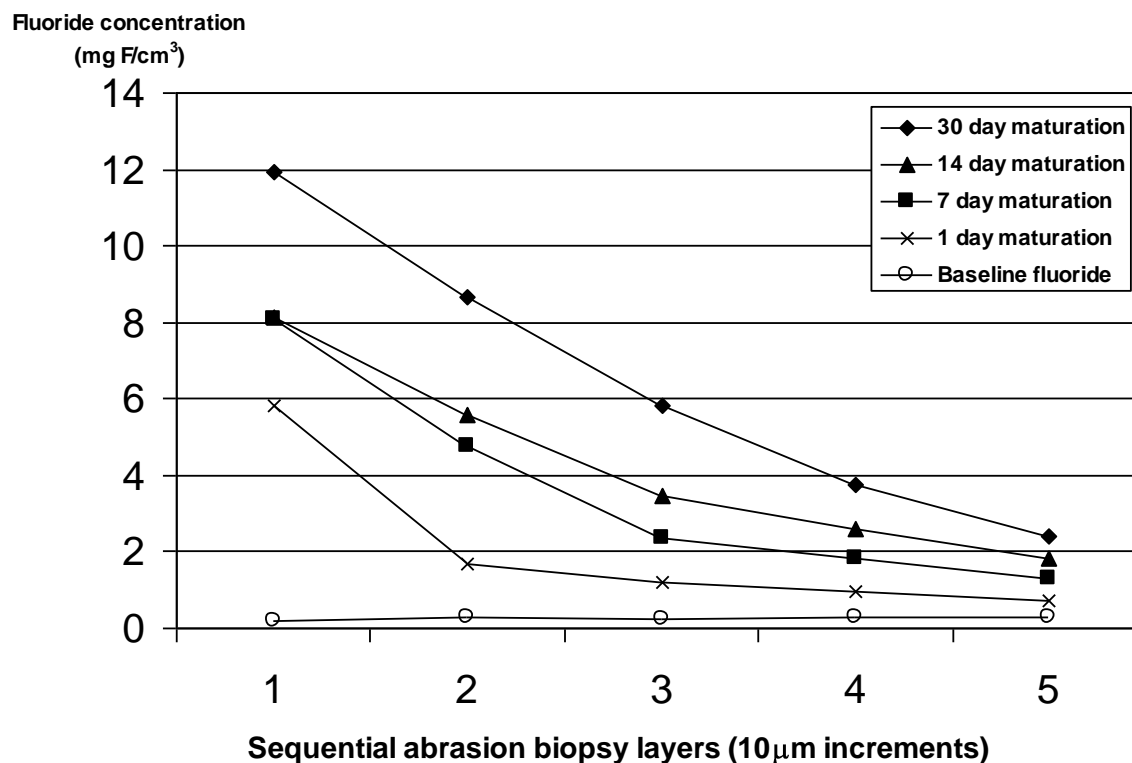
Figure 7. Fluoride concentration measured from the Ketac-Fil/dentin interface after various maturation periods.[2]

A comprehensive study by Francci et al. (1999) investigated fluoride release from many restorative materials and the effect on dentin demineralization.[3] In the study, 144 extracted bovine teeth were sectioned to the crowns, facial enamel was removed, and lingual surfaces ground to the pulp chamber. The teeth were then sectioned longitudinally into halves and the pulp chambers and lingual surfaces covered with bonding agent and light activated. Each crown half was then glued to a slide using cyanoacrylate, and the facial dentin prepared to 1.8 mm diameter x 0.8 mm depth. Teeth were then restored with one of 6 protocols: 1) Single Bond & Z100, 2) Single Bond and Tetric Ceram, 3) Fuji Bond LC & Z100, 4) FujiBond LC & Tetric Ceram, 5) Fuji II LC, and 6) Fuji IX GP. Single Bond is a non-fluoride-releasing material, while Fuji Bond LC is reported by the manufacturer to release fluoride. Z100 does not contain fluoride, while Tetric Ceram contains and is reported by the manufacturer to release fluoride. Fuji II LC is a RMGIC, and Fuji IX GP is a conventional glass ionomer. Specimens were stored in 37°C water for 20 minutes, polished with 600 grit SiC paper for 30 seconds to simulate

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restoration finishing, and either subjected to lactic acid challenge, or bacteria (S. mutans exposure) challenge.

For the dentin demineralization/lactic acid challenge experiment, seventy-two of the specimens (half) were placed in wells with 30 μL of deionized water. At 24 hours, the solution was removed, and the reservoirs filled with 60 μL lactic acid solution at pH 4.3. After 3 hours, the wells were sampled for Ca^{2+} ions to measure calcium release from the teeth. The other half of the specimens, used in a S. mutans challenge experiment, were placed similarly in 30 μL of deionized water for 24 hours, then 30 μL of Todd-Hewitt Broth with S. mutans NCTC 10449 along with 50 mM glucose (0.54 μg) were added to each well and allowed to incubate for 6 hours at 37°C. At the end of the 6 hours, 30 μL was retrieved and calcium ions assayed. As one further test, each material listed above was measured for fluoride release by fabricating 6 mm diameter x 2 mm height discs. Each material was placed in 60 μL deionized water, and stored for 24 hours. Thereafter, 20 μL samples were taken and fluoride concentration measured using a fluoride ion-specific electrode.

Results of the various testing showed that fluoride release was highest for Fuji Bond LC (32 μg), followed by Fuji IX GP (18 μg) and Fuji II LC (12 μg). Single Bond, Tetric Ceram, and Z100 had minimal (<1 μg) fluoride release over 24 hours in deionized water. For specimens subjected to the lactic acid challenge for 3 hours, Fuji IX GP released the most fluoride into solution (fluoride release into tooth was not measured) with 144 ± 68 ng, followed by Fuji II LC (26 ± 14 ng) and the combination of Fuji Bond LC & Tetric Ceram (11 ± 17 ng). The combinations of Single Bond with Z100 or Tetric Ceram yielded no measurable fluoride into solution. Fuji Bond LC and Z100 also yielded no measurable fluoride release. Adjusted calcium release measurements from the lactic acid challenge showed an inverse relationship to fluoride release, with only Fuji II LC (137 ± 17 ng) and Fuji IX GP (110 ± 11 ng) limiting calcium release (and thus dentin demineralization) to any significant amount less than about 160 ng. Finally, specimens subjected to S. mutans challenge for 6 hours demonstrated calcium release as follows: Single Bond & Tetric Ceram (277 ± 104 ng) > Fuji Bond LC & Tetric Ceram (214 ± 47 ng) ≈ Fuji Bond LC & Z100 (212 ± 31 ng) ≈ Single Bond & Z100 (199 ± 71 ng) > Fuji II LC (182 ± 76 ng) > Fuji IX GP (116 ± 35 ng). The results of this study indicated that glass ionomer restoratives such as Fuji IX GP had more fluoride release than resin-modified glass ionomers

(Fuji II LC). However, RMGICs had significantly more fluoride release than fluoride-containing composites such as Tetric Ceram or fluoride-containing bonding agents (Fuji Bond LC).

In a multi-faceted study of various restorative materials, Nagamine et al. (1997) investigated the effect of RMGICs on secondary caries.[37] Using Teflon plastic molds, Fuji II LC, Photac-Fil, Vitremer, Fuji II, and Z100 restorative specimens were fabricated and light cured (except for Fuji II) for 80 seconds. Five specimens were fabricated per material. All specimens were immersed in 8 mL of water at 37°C for time periods ranging from 2-161 days. Water was replaced after each test period, buffered, and measured with a fluoride ion-specific electrode. Additionally, 32 extracted premolars were prepared with Class V cavities into dentin, restored with Fuji II on the buccal, and with one of the three RMGICs or Z100 on the lingual. Teeth were stored at 37°C in water, polished after 24 hours, then stored for an additional 28 days. Subsequently, specimens were coated with nail varnish except for the 0.5 mm surrounding area around the restorations, and subjected to an artificial caries solution of 0.5% yeast extract and 1% sucrose inoculated with S. mutans at 37°C. The medium was replaced every 3-4 days. After 20 days, specimens were removed from the solution, mounted and sectioned into 50-60 μm thicknesses, and examined using microradiography. Depths of lesions created by the carious attack were measured at sites 50 μm apical to the gingival margin as well as the acid-resistant layer adjacent to the gingival wall 300 μm beneath the facial surface of the restorative. Also, electron microprobe analysis was done from one section in each group. At a distance of 300 μm beneath the restorative facial surface, a linear analysis was performed at 15 kV and 2 x 10⁻⁸A. Results of the experiments are shown in Figure 8. As seen in Table 3, Photac-Fil had the most fluoride released after 2 days, followed by Fuji II, Vitremer, and Fuji II LC. Cumulatively, all the resin-modified glass ionomers (Photac-Fil, Fuji II LC, and Vitremer) showed as much fluoride release as the pure glass ionomer (Fuji II).

Additionally, the mean depth of the outer lesion was measured. Mean depths ranged from 742 μm (Z100) to 309 μm (Fuji II). Similar to the glass ionomer, the RMGICs had average lesion depths of 331-380 μm. The average acid-resistant layers ranged from 0 (Z100) to 55 μm (Fuji II), with the RMGICs ranging from 34-46 μm. Finally, in the electron microprobe line analyses, all gingival walls adjacent to fluoride-containing restoratives measured high in both calcium and fluoride, with no difference in profiles between RMGICs and the GIC (Fuji II). For

Z100, no fluoride was detected and calcium content had decreased next to the restorative due to the carious attack.
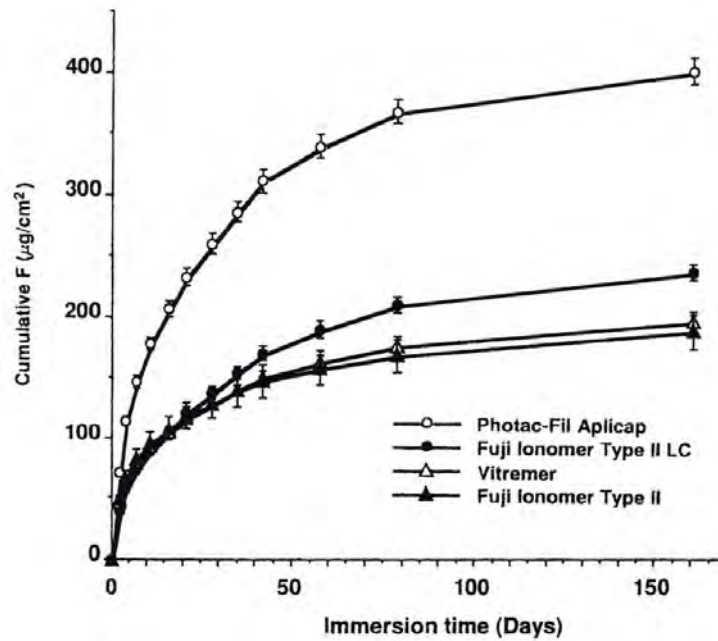
Figure 8. Average cumulative fluoride release from various GIC and RMGIC restoratives.[37]

Table 3. Cumulative Fluoride Released After Immersion in Water for 2, 28, and 161 days.[37]

Immersion Time	$\mu g/cm^2$ fluoride released (cumulative)			
	Photac-Fil	Fuji II LC	Vitremer	Fuji II
2 Days	71	39	46	49
28 Days	260	138	129	129
161 Days	400	236	196	188

Nanohardness and microhardness studies of dentin

As previously discussed, one method to evaluate the release of fluoride into dentin is to measure the ability of the material to inhibit demineralization in the dentin. In many studies already reviewed, demineralization experiments were performed on dentin to measure areas or zones of inhibition.[2,3,32,36,37] Additional studies have concentrated on using techniques such as

microhardness measurements to identify the differences in hardness resulting from dentin demineralization. Subsequent to a demineralization episode, hardness measurements can be made to determine the resistance of dentin to dissolution of calcium and phosphorus ions. In fact, Davidson et al. (1974) reported that microhardness can be measured as a linear function of calcium content.[38] A similar correlation has been demonstrated by Featherstone et al. (1983).[39]

An examination of the microhardness of *normal* dentin and enamel is important before investigating how demineralization episodes can change the physical properties. Research by Meredith et al. (1996) concluded that the hardness of dentin increased as the distance increases from the dentinoenamel junction.[40] Also, hardness in enamel was measured to decrease significantly with distance from the tooth surface. In the study, three permanent molar teeth were embedded in resin and cut buccolingually into 2 mm slices perpendicular to the mesiodistal axis of the tooth. Specimens were then polished down to a 1 μm finish and microhardness testing done 20 times each in enamel and dentin using a Knoop indenter. For each indentation sequence, indents were arranged in three rows of five, starting 300 μm into enamel and 500 μm into dentin from the DEJ. Indents were spaced by 300 μm in enamel and 500 μm in dentin. Loads of 0.98 N in dentin and 4.9 N in enamel were utilized. These loads caused indentation long diagonals of about 100 μm in dentin.
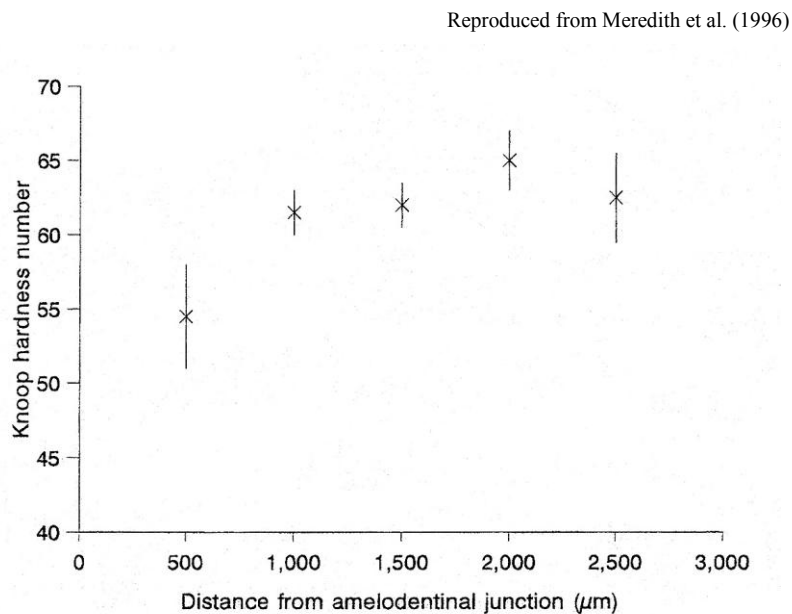
Reproduced from Meredith et al. (1996)

Figure 9. Mean Knoop hardness in dentin as a function of distance from the DEJ.[40]

Results of the testing showed a 4-9% error possibility in calculations of hardness, which were measured using the long diagonal of the indents. In fact, both hardness and Young's moduli were calculated using formulas. Figure 9 shows the hardness from the DEJ inward toward the pulp. Although the changes seen in Figure 9 are gradual processes that would not be expected to manifest significantly within a 100 μm distance as planned in the research proposal, this is an important study reviewing a hardness measurement technique. Clearly the 100 μm indentation size is far too large for the proposed research, as the indentation technique used in this research involved Knoop testing which is more on a macro-scale. Additionally, certain nanoindenter instruments are able to automatically calculate hardness and Young's modulus during the indentation procedure, not relying on any inaccuracy from hand measurement of diagonal lengths.

It is also important to review some studies that have employed hardness testing to measure differences in demineralized or carious lesions versus normal dentin. For instance, Marshall et al. (2001) investigated the nanomechanical properties of carious dentin.[41] In the study, eight occlusal carious teeth and 3 non-carious third molars (controls) were used to prepare 1-2 mm sagittal slices, which were then polished to a 0.25 μm finish. An atomic force microscope (Nanoscope III) was used right after final polishing to make indentations into the sections. A 90° diamond tip was used for indentations, with loads between 100-500 μN at 3 seconds each. At intervals of less than 200 μm from the pulp to the carious occlusal area, clusters of 10 indentations were made. Also, areas of peritubular and intertubular dentin were individually indented. Results showed that the moduli and hardness values increased slightly from the pulp into normal dentin.[41] The values then decreased in carious dentin. Mean elastic moduli and hardness values for visibly normal intertubular dentin and the controls were not significantly difference. However, the average modulus (18.2 ± 3.2 GPa) and nanohardness (0.8 ± 0.2GPa) for transparent intertubular dentin—an affected zone under discolored carious dentin—was significantly lower than that of intertubular unaffected dentin (modulus = 20.6 ± 2.2 GPa and nanohardness = 1.0 ± 0.1 GPa). For many areas of the lesion though, there was no significant difference, which indicated the dentin was unchanged in the transparent zone. Peritubular dentin in the transparent zone and normal, unaffected areas had elastic moduli of 36 and 39 GPa, respectively. Nanohardness measurements were 1.9 and 2.8 GPa for the transparent zone and normal, unaffected areas of dentin, indicating significant differences between affected

dentin and unaffected dentin. The results of these experiments show that accurate nanoindentation testing can be performed on dentin and that the presence of dentinal tubules will not affect the overall measurements. Additionally, Craig and Peyton (1958) were unable to substantiate the supposition that dentin hardness is dependent on specimen orientation.[42] This current study reinforced other research claims that the intertubular dentin is a good representation of the dentin as a whole; the peritubular dentin has only a small contribution to the properties of the dentin. Intratubular (peritubular) mineral deposits did not appear to significantly contribute to the mechanical properties of dentin. Therefore, nanoindentation procedures may be done on intertubular dentin and projected as a good representation of the overall dentinal hardness.

In an article comparing different techniques for evaluating demineralization, Featherstone et al. (1983) used quantitative microradiography and microhardness to assess artificial caries-like lesions in enamel.[39] In the study, seven crowns were sectioned from the roots and coated with nail varnish, leaving two small windows. The specimens were immersed in a variety of buffer systems including 0.1 M lactic acid, 0.1 or 0.2 mmol MHDP and pH values of 4.5 or 5.0. Length of demineralization immersion was either 3 or 14 days. Subsequently, the crowns were cut vertically in half, using one half for microradiography and the other for microhardness profiling. Quantitative microradiography was done on 80 μm thick sections ground down to 1200 grit. A Leitz microscope-photometer (MPV) was used, with the beam traveling at a rate of 3 μm/sec. Mineral content of enamel at 1 μm intervals was calculated. Microhardness profiles were created by polishing tooth halves to a 1 μm finish and measuring with a Knoop diamond indenter at 25 μm intervals from the demineralization lesion into sound enamel. Three positions, at ¼, ½, and ¾ along the width of the lesion were profiled for hardness. Fifteen and 50 g loads were used for indentations.

The results from Featherstone et al. demonstrated a correlation between the inverse of indentation length (or Knoop hardness^{½}) and volume percent mineral within the enamel. In fact, an equation could be fit to the regression line, with r = 0.916. The data, according to the authors, holds to the equation for demineralized enamel in the region of 40-90% mineral by volume. Additionally, they report that the relationship also holds for sound dentin. However, they did not provide data to support this claim. In conclusion, the research demonstrated that microhardness

measurements can be used as a direct measure of mineral gain or loss due to de- or re-mineralization.

A contrasting study by Akimoto et al. (2001) aimed to measure nanohardness of dentin after acid etching and restoring with composite.[43] In the study, in vivo remineralization was measured using nanoindentation and EDS instruments after 7 days and a 6-month period. Rhesus monkey teeth were restored with 20 Class V restorations in vivo, with depth measuring about 1.5-2 mm. Teeth in were all etched for 60 seconds with 37% phosphoric acid and primed using SA-Primer. For the 10 teeth in group 1, Protect Liner (a resin liner) was applied to all walls and light cured. Clearfil AP-X composite was then used to restore the preparations. Teeth in group 2 were lined with Photobond adhesive and light cured, followed by Protect Liner on the axial floor and restored with Clearfil AP-X composite. The Rhesus monkeys were sacrificed, with half the teeth from each group tested 7 days after restoring, the other half after 6 months maturation intraorally.

Nanoindentation samples were made by cross sectioning the teeth perpendicular to the resin-dentin interface through the center of the cavity. Samples were then polished down to 0.25 μm grit, ultrasonically cleaned, rinsed, and dried. Using an ENT-1100 Nano Indentation Tester (Elionix), five specific areas across the resin-dentin interface were evaluated; nanoindentation imprints were made in the areas of the Protect liner, demineralized dentin area, Photobond layer, resin-impregnated layer, and normal dentin. A 100 mgf load was used and SEM utilized to observe each impression. Finally, EDS was used to measure Ca content across the interface of some of the tooth specimens. Results of the testing for group 1 showed no statistically significant differences in the nanohardness of demineralized dentin (caused by acid etching) and 5 μm away from the demineralized area at 7 days. Similarly for group 2, there was no difference between the resin-impregnated layer and dentin 5 μm away. For group 1 there were no differences in the demineralized areas at 7 days compared to 6 months, but the area 5 μm away from the demineralized area had increased from 52.7 ± 18.8 to 83.1 ± 19.4 mgf/μm^2. Similarly, for group 2 at 6 months, the hardness 5 μm from the resin-impregnated layer area had increased significantly from 53.8 ± 18.8 to 81 ± 25.4 mgf/μm^2.

Group 2 restorations exhibited a non-resin impregnated hybridoid layer, which has been reported to have just 60% of the nanohardness of sound dentin due to demineralization. Indeed, EDS analysis results showed that at 7 days, Ca levels in the group 2 hybridoid layers had

decreased Ca levels. At 6 months though, Ca levels in the hybridoid layer had reached the level of normal dentin, indicating slow remineralization. The methodology and results of Akimoto's study demonstrates that nanoindentation can be done accurately at tooth-restorative interfaces with different materials and even different areas of demineralization. With the proposed research including the use of fluoride to decrease the effects of a planned demineralization episode, Akimoto's study is proof that it may be possible to measure hardness differences that correspond to different pre-demineralization amounts of fluoride.

Comprehensive studies of fluoride release and demineralization inhibition

Research specifically focused on measuring the effect of fluoride on demineralization inhibition using microhardness or nanoindentation is fairly limited. Even more rare are studies using microhardness to measure demineralization inhibition due to glass ionomer-based fluoride release. One such study was published by Kotsanos (2001), who investigated the effect of glass ionomer restorations on demineralization inhibition.[44] In the research, the carious inhibition effect of three fluoride-releasing restorative materials on sound enamel was measured using microhardness testing. In the study, bovine enamel slabs measuring 3 x 2 mm were prepared, ground flat and polished. Two rows of indentations were made for initial microhardness measurements using a Kentron microhardness tester and 200 g load. Initial indentations measured almost 100 μm, penetrating about 3 μm into enamel. Four restorative materials, including Pertac II (non-fluoridated composite), F-2000 (compomer), Ketac Molar (glass ionomer), and Vitremer (resin-modified glass ionomer), were used to prepare hemispherical samples measuring 4 mm in diameter. The hemispheres were arranged so the curved surface contacted an enamel slab at one contact point at the center, and there was free space for plaque accumulation.[44] Two human volunteers were then equipped with mandibular complete dentures with wells placed in the buccal flanges with the enamel-restoration pairs attached using cold-cure acrylic. The subjects then wore the dentures continuously for 70 days, without brushing the experimental area and using a non-fluoridated dentifrice. With this regimen, the experimental areas were covered in plaque except for the contact areas between the bovine slabs and restoration hemispheres. After the experimental period, the enamel slabs were removed and indented as before to measure microhardness.

Figure 10 shows the differences between the four restorative materials, and the dependence on distance from the contact point. Significant differences were found between all restorative materials, with Vitremer providing the best inhibition of demineralization, and Pertac II providing the least inhibition. As distance from every restorative material increased, microhardness decreased, indicating less demineralization resistance. Average inhibition was 86% for Vitremer, 69% for Ketac Molar, and 42% for F-2000 in relation to Pertac II. Polarized light microscopy showed no subsurface lesion in the slabs adjacent to Vitremer, while those next to Ketac Molar and F-2000 had small lesions, and Pertac II had the clearest subsurface lesions. The results of this experiment were fairly consistent with those found in other tests of fluoride release. The theory that fluoride release can be measured by microhardness is well demonstrated with this study.
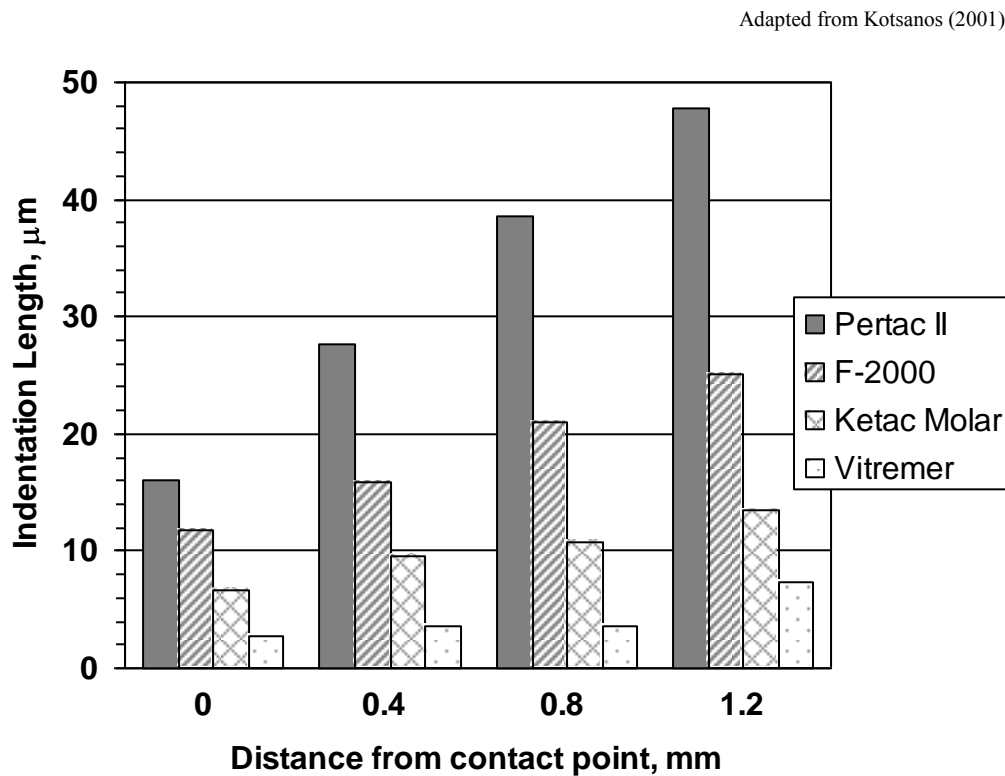
Adapted from Kotsanos (2001)

Figure 10. Microhardness of dentin as a function of distance from contact with a restorative.[44]

A pair of more complete investigations on the effects of fluoride were published by Shibatani et al. (1989). In a two-part study, the transfer of fluorine from a fluoridated resin to

dentin was measured, followed by a measurement of the acid resistance of the dentin.[45,46] In part 1 of the study, two types of composite resin, F-resin and C-resin were used. The F-resin contained a fluoride-releasing copolymer of methacryloyl fluoride and methyl methacrylate and the C-resin was a non-fluoridated control with otherwise similar components to the F-resin. Two concentrations of the F-resin were prepared: a 0.46 molarity fraction and a 0.33 molarity fraction. Five discs measuring 1 mm thick and 20 mm in diameter of each of the fluoridated resins (0.46 F-resin and 0.33 F-resin) were prepared and placed in phosphate buffer for 560 days with intermittent replenishing of the solution and measurement of released fluoride ion. Additionally, fluoride uptake distribution in dentin of the F-resins and the C-resin was measured. A flat dentin surface was produced through grinding non-carious premolar tooth roots with 1000 grit paper, parallel to the root surface. Cylindrical resin specimens of 3.2 mm diameter x 2 mm height were cured onto the dentin specimens and the whole sample covered with dental wax. All specimens were then incubated in 1 mL of 0.2 M phosphate buffer (pH 7.0) up to 180 days, with intermittent exchange of buffer. Following incubation, the specimens were cut perpendicular to the resin-dentin interface using a low-speed cutter and the surface polished. Finally, linear analysis of Ca, P and F was done using a Shimadzu EPMA-8705 electron probe microanalyzer. WDX microanalysis settings were 15 kV, 50 nA, a 1 μm beam size and sample speed of 20 μm/min.

Results of the tests demonstrated that the fluoridated resins (F-resin) released fluoride in solution as well as into dentin. Initial aqueous release for the 0.46 F-resin was 30.4 μg/day and rapidly decreased to 13.7 μg/day after 6 days, down to 5.6 μm by day 32 and 1.0 μg/day by day 540. Release of fluoride from the 0.33 F-resin was about half of that of the 0.46 F-resin. Measured release was 12.4 μg/day initially, which decreased down to 5.4 μg/day after 6 days, 2.6 μg/day by day 32, and 0.3 μg/day by day 540.

WDX microanalysis at 0 days of incubation (no immersion in phosphate buffer) demonstrated a 14-15% fluoride level above the fluorapatite standard at 5 μm into dentin which decreased rapidly down to baseline values at 20 μm. After 1 day of incubation, a similar trend was seen, although the initial fluoride amount was 30-40% of the standard. At 7 days of incubation, surface fluoride was up to $46 \pm 20\%$ of the standard, decreasing slightly in the initial 20-30 μm, and finally dropping significantly to baseline by 30-50 μm. At 30 days of incubation,

surface fluoride was $55 \pm 7\%$ of the standard, and dropped significantly within 100 μm of the interface. At 90 and 180 days of incubation, the decrease in fluoride as a function of time was very gradual. Surface concentration had slightly decreased when compared to the 7 and 30-day incubated specimens, while the depth of penetration was 120 μm at 90 days and 180 μm at 180 days (where baseline fluoride measurement was reached). The authors reported that X-ray diffraction of some 90-day F-resin treated specimens showed that the increase in fluoride occurred through the incorporation into hydroxyapatite crystals to form fluorohydroxyapatite. The overall conclusions of the first part of the research were:

1) The initial phase of fluoride release is short, and a large amount of fluoride is released. This is likely due to release of free fluoride (unbound or from immediate surface dissolution).
2) Fluoride release was maintained in the superficial 50 μm layer of dentin, only migrating slowly to deeper areas.
3) Fluoride penetration was measurable up to 180 μm into dentin after 180 days of incubation.

Some shortcomings arise with the first part of this 2-part study, including: 1) the fluoride concentration of the fluorapatite standard from which the WDX relative fluorine concentrations were measured was not reported, 2) the detection limit (in ppm or wt. % fluorine) of the WDX microanalysis was not reported, 3) no apparent specimen degradation was reported, which identifies if the dentin was being damaged (and thus fluorine counts diminishing over time) as the specimen was being analyzed, and 4) there was no demonstration of the method used to determine the start of the tooth-restorative interface. Another one of the identifiable shortcomings of this study is that a composite resin restorative material was used. In the United States, composite resin materials are typically placed after phosphoric acid etching followed by bonding agents. As mentioned previously and demonstrated by many studies, fluoridated composite resins will release minimal fluoride directly into dentin when placed with a bonding agent barrier. Furthermore, even fluoridated bonding agents are inhibited from releasing significant amounts of fluoride unless microleakage at the tooth-bonding agent interface has occurred.

The second part of the study by Shibatani et al. (1989) aimed to study the enhancement of acid resistance of the dentin from fluoride uptake.[46] Using the same method of preparation for

dentin/resin specimens, each root from 36 premolars was ground using 1000 grit paper and divided into three blocks. One block was restored with F-resin, one with C-resin, and the last one left untreated. Resin restorations measuring 2 mm in height and 3.2 mm in diameter were placed onto the dentin surfaces. Specimens were incubated in 5 mL of 0.2 M phosphate buffer (pH 7.0) for 0, 1, 7, 30, 90, and 180 days. Upon completion of incubation, the resin was removed from each specimen and the entire specimen coated with wax except for a window of 2 mm diameter where the resin had been in contact with the dentin. Subsequently, 1 mL of 0.2 M acetic acid-acetate buffer (pH 4.5) at 37°C was used to demineralize for periods of 0.5, 0.5, 1.0, and 1.0 hours (total of 3 hours). Calcium ion concentration was measured using an Ionalyzer fluoride-specific electrode. After demineralization, the wax was removed, the specimens sectioned perpendicular to the exposed surface, and Knoop hardness measured every 25 μm to a depth of 300 μm into the dentin.

Results of the testing demonstrated that the C-resin (non-fluoridated) did not inhibit calcium dissolution from the acetic acid-acetate buffer, while the F-resin significantly decreased the amount of dissolution, up to over 60% (compared to the non-treated control) after a 180 day incubation period. Even at 0 days of incubation in phosphate buffer, there was about 20% inhibition in calcium dissolution after 3 hours in acetic acid for the F-resin treated specimens. After 7 days of incubation, the inhibitory effect from F-resin was 63, 48, 41, and 37% for 0.5, 1, 2, and 3 hours of demineralization exposure, compared to controls.

The reduction in Knoop hardness after 3 hours of decalcification for the F-resin specimens was much more evident in superficial parts of the specimen (near the surface). In fact, at 100, 150, and 200 μm, there was no significant difference in hardness. After 0 and 1 day of incubation, Knoop hardness was unchanged in the F-resin specimens compared to controls. By day 7, hardness at 25 and 50 μm depths was 7 and 25 for the F-resin specimens, compared to about 3 and 10 for the C-resin and control specimens. By day 30, the 50 μm hardness of F-resin specimens was 30, compared to about 10 for the controls.

In this study by Shibatani et al., acid resistance was measured as a function of depth from the demineralization insult. Additionally, the dependence on acid-exposure time and fluoridated resin exposure time was determined. While the first part of the 2-part study measured fluoride penetration using an electron microanalysis and the second part measured acid resistance, different specimens were used for each experiment. In the acid resistance study, the specimens

were exposed to demineralization solution where the resins had been in contact with the dentin. Subsequently, the specimens were sectioned perpendicular to the demineralized surface, testing the penetration depth of the demineralization. In the proposed research, this sequence is slightly different, as the surface exposed to the demineralization episode will the same surface measured for nanohardness. Thus, the measurements will determine the resistance to demineralization solely as a function of fluorine concentration within the dentin, not depth from the direct contact with demineralization solution.

PRELIMINARY STUDIES

A pilot study to determine the viability of the proposed research was done at the University of Michigan School of Dentistry. This study involved extracted first and second maxillary and mandibular molar teeth, with Class V cavity preparations restored with Fuji II LC. After 1, 2, 5, 10, 15, and 20 days of immersion in 37°C distilled, deionized water, the specimens were sectioned horizontally, mounted in acrylic resin, polished, and examined using electron microprobe analysis.

A Cameca SX100 Electron Probe Microanalyzer was calibrated and set to a 5 kV excitation voltage, 10 nA, and a 1 μm beam focus with each point analyzed for 5 seconds. Settings of the microprobe were maintained for all specimens analyzed. Analyses of about 50 μm in length from the tooth-restorative interface were conducted for 1, 2, 5, 10, 15, and 20 day matured specimens. Two to four analyses per specimen were made, with two specimens for each immersion time period. Raw count data was collected for each analysis and averaged. Figure 11 shows the typical appearance of the junction between dentin and Fuji II LC seen during the process of measuring for fluorine. The differentiation between tooth and restorative material are clear. The left side of the photo represents the resin-modified glass ionomer Fuji II LC. The dark areas are the fluoroaluminosilicate glass particles, and the lighter areas the polyacrylic/resin matrix. On the right side of the photo is the dentin, with dentin tubules sectioned at an oblique angle. At a higher magnification, the interface appears as a small gap, which usually measures 5-10 μm. Such a gap may be due to specimen preparation, with hardness differences in materials (dentin vs. restorative) causing a "gap" to appear after polishing. More likely, the gap is due to desiccation of the dentin or the glass ionomer restorative, causing interface debonding.
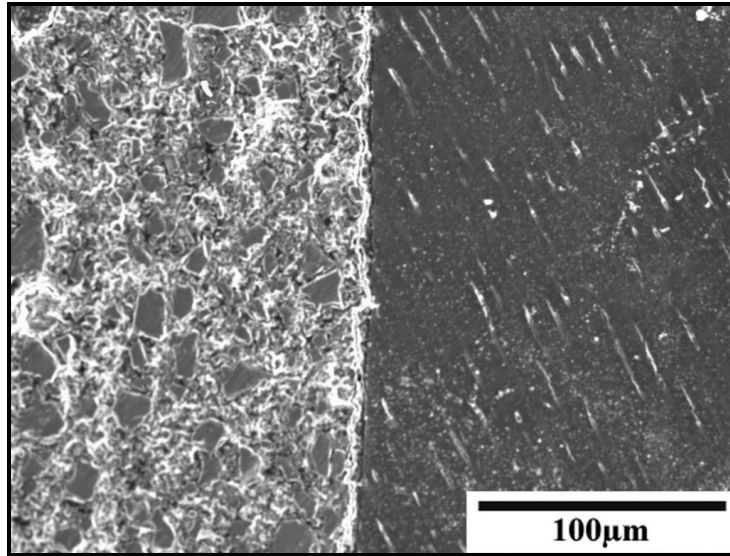
FIGURE 11. SEM photo demonstrating the interface between Fuji II LC (left) and dentin (right) from a sectioned Class V restoration.

Figure 12 shows the plot of fluorine raw counts vs. distance from the tooth-restorative interface. It can be seen that over the first 20 μm from the interface, the relative fluorine levels are elevated from the baseline. The trend for all maturation days appears similar, with fluorine levels dropping off significantly from the interface. With glass ionomers, studies have found a high initial rate of fluoride release that quickly declines. This represents surface dissolution of fluoride ions that occurs primarily during material setting. A shallower fluoride release curve that steadily declines will indicate fluoride diffusion, which is a slow process. Perhaps both phenomena are occurring at the tooth-restorative interface with Fuji II LC. The high level of fluoride uptake at the first 10-15 μm of the dentin after day 1 would appear to indicate a surface dissolution phenomenon. After many days, however, only a slow diffusion process is occurring, therefore showing very similar curves compared to day 1. The data demonstrates that the 1, 2, and 5 day specimens appear to differ significantly from the 10, 15, and 20 day specimens in regard to the baseline levels and overall fluorine counts. This difference may be attributed to instrument variation, as the 1, 2, and 5 day specimens were all analyzed on the same day, while the 10, 15, and 20 day specimens were analyzed together on a different day. Therefore, slight differences in instrument calibration (drift) will lead to the different raw counts. For all the curves in Figure 12, a baseline level is reached at about 20 μm in from the interface. This fluorine level is most likely the detection limit of the instrument. Calculations from quantitative

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measurements indicate that the detection limit for fluorine with the instrument conditions of 5 kV excitation voltage, 10 nA, and each point analyzed for 5 seconds was between 1,000-1,800 ppm, depending on the point analyzed. Therefore, the trends seen in Figure 12 in the first 20 μm from the tooth-restorative interface appear legitimate, with ppm of fluorine calculated to be above about 1,800 ppm. A method of determining actual ppm can be done by lowering the detection limit through altering instrument conditions. As described in many textbooks, a longer counting time per analysis point can lower the detection limit.[47] Therefore, it was expected that by altering instrument settings such as analysis time, a lower detection limit for fluorine will be achieved and more noticeable differences could be seen between the different maturation times.

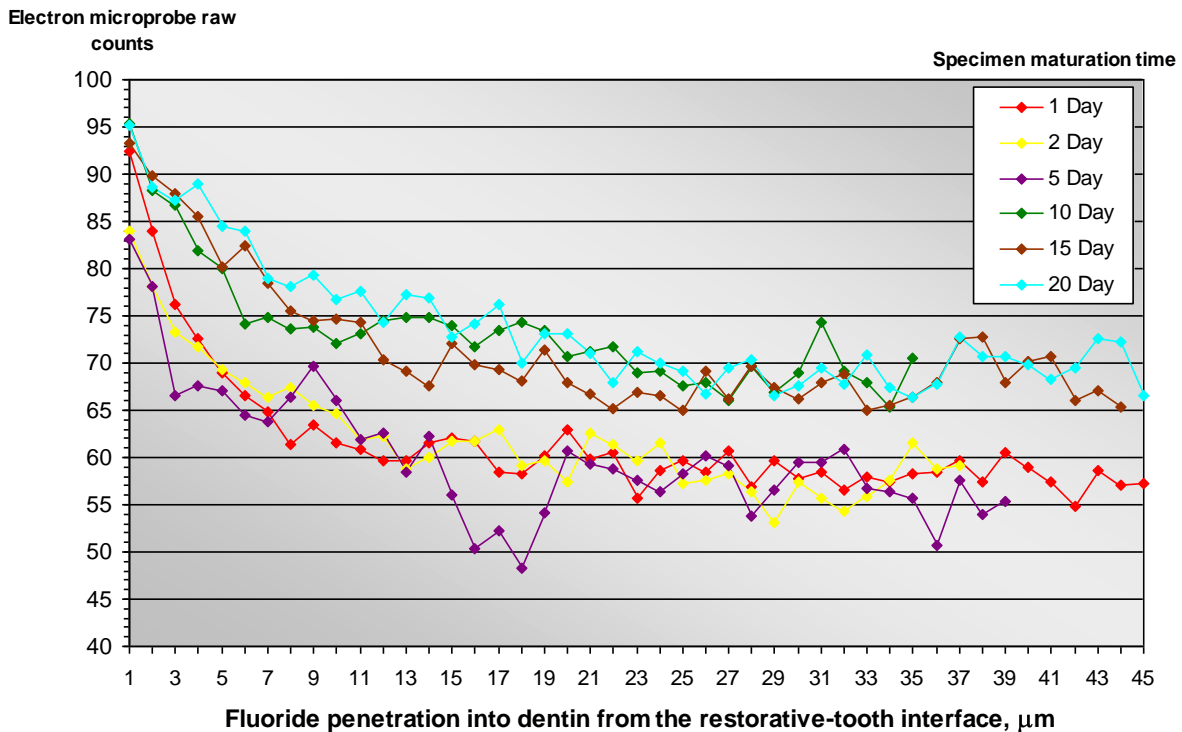FIGURE 12. Average fluoride raw counts measured from the tooth-Fuji II LC interface after 1-20 days of specimen maturation.

In a continuation of the pilot study, electron microprobe settings were adjusted to achieve a lower detection limit. Additionally, adjustments to the software settings made it possible to directly calculate and plot weight percentages of the elements, rather than simply the raw counts.

With the adjusted electron microprobe settings, analysis of individual points was performed on a calibration standard containing 3.67% fluorine as well as on dentin specimens. This procedure was done to monitor the elemental X-ray counts to ensure that the measured fluorine levels did not dissipate and minimal specimen degradation occurred through the duration of the point analysis.

FIGURE 13. Average calcium raw counts measured from the tooth-Fuji II LC interface after 1, 10, and 20 days of specimen maturation.

Glass ionomers bond to teeth through an ionic bond. This bond is enhanced through pretreating cut tooth surfaces with polyacrylic acid prior to restoring. Recommended by the manufacturer prior to placing Fuji II LC, GC cavity conditioner contains 20% polyacrylic acid and will remove the smear layer for better tooth-restorative bonding. Such conditioners may also have an effect on the dentin's superficial mineral content. The act of dentin conditioning prior to placing glass ionomer and resin-modified glass ionomer restorations may be accompanied a localized decrease in calcium and phosphorus concentration at the tooth-restorative interface. In fact, a decrease was seen in the initial approximately 5-10 μm of dentin in the plots of calcium

and phosphorus as demonstrated by some of the plotted calcium counts in Figures 13 and 14. The microprobe results from control specimens restored with Valiant Ph.D. amalgam, without the use of cavity conditioner should help determine whether or not the 20% polyacrylic acid cavity conditioner may be responsible for the changes the calcium and phosphorus at the interface.
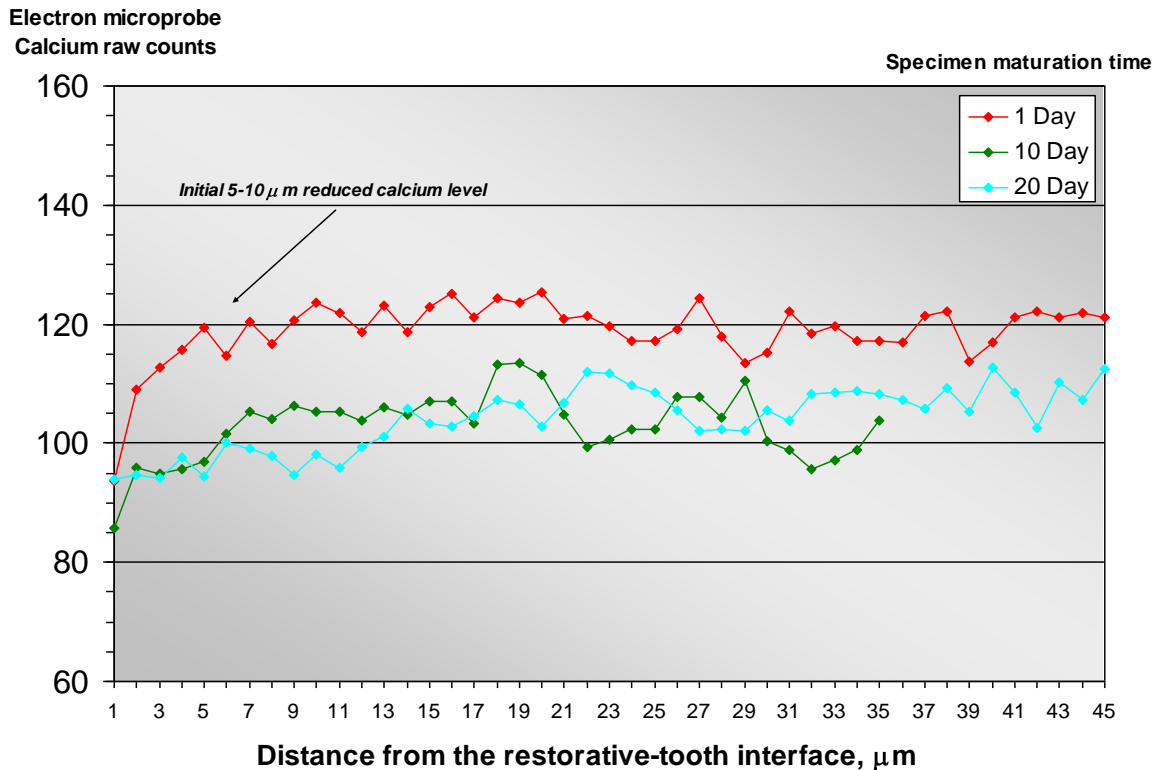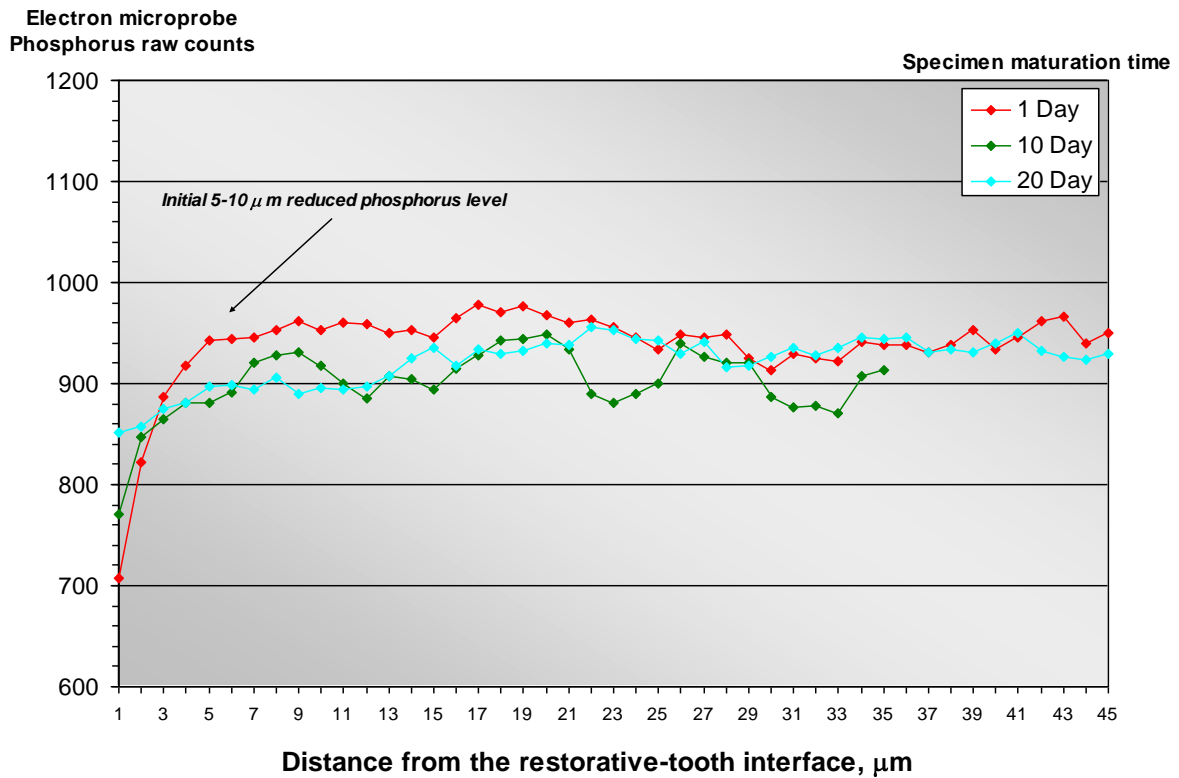

FIGURE 14. Average phosphorus raw counts measured from the tooth-Fuji II LC interface after 1, 10, and 20 days of specimen maturation.

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CHAPTER 2

Time-dependent fluoride uptake into dentin from a resin-modified glass ionomer

ABSTRACT

Objectives. The objective of this study was to use electron microprobe analysis to measure the amount of fluoride uptake and the depth of penetration into dentin from a resin-modified glass ionomer material (Fuji II LC) at different time intervals after restoration placement. Additionally, the electron microprobe specimens were subjected to a demineralization challenge and the nanohardness and Young's moduli measured in the dentin in an attempt to correlate fluoride concentration to demineralization resistance.

Methods. Intact human molars were disinfected, prepared, and restored with Class V restorations using either Valiant Ph.D. (control) or Fuji II LC. The samples were matured in a 100% humidity environment for 1, 15, or 30 days, at which time they were mounted and sectioned. A linear analysis of the dentin fluoride concentration and depth penetration from the axial wall of each specimen was performed using a WDX electron microprobe. Each specimen was then subjected to a 0.1M buffered acetic acid demineralization challenge and tested for nanohardness and Young's modulus from the axial wall interface inward using a Nanoindenter XP instrument. Microprobe results were evaluated using a 1-way ANOVA with $\alpha = 0.05$ at each distance interval from the tooth-restorative interface.

Results. Electron microprobe analysis demonstrated negligible fluorine content throughout the dentin for the amalgam control group. For Fuji II LC restored specimens, an average interfacial fluorine level of 3,800 ppm was seen for the 1 day group, which dropped below the detection limit within the first 10 μm. However, the 15 and 30 day groups had fluorine penetration above the detection limits up to 20-30 μm into dentin, with interfacial fluorine levels almost identical, at around 5,600 ppm. ANOVA demonstrated no significant differences between the control and 1 day-matured groups. For the 15 day specimens, there was significantly more fluoride detected in dentin within the first 12 μm compared to the controls. Likewise, for the 30 day specimens, there was a significant difference in fluoride within the first 16 μm compared to controls. Similar differences were seen between the 1 day specimens compared to the 15 and 30 day specimens. Few significant disparities in dentin fluoride content were shown between the 15 and 30 day specimens and there were no significant differences among any of the specimens noted beyond 24 μm. Demineralization and nanoindentation results showed no clear and explicable differences between most of the specimens, including undemineralized controls. The demineralization sequence or the nanoindentation technique used to measure the mechanical properties may not have been sensitive enough to detect the impact of fluoride on the resistance to acid attack.

Significance. Electron microprobe analysis demonstrated significant concentrations and penetration of fluorine in dentin, released from a resin-modified glass ionomer restorative after 30 days of in vitro maturation. A correlation between measured fluoride concentration in dentin and resistance to demineralization could not be established using the chosen demineralization sequence and nanomechanical testing. Further studies are needed to show the long-term fluorine diffusion and correlate specific concentrations of fluoride in dentin with the ability to resist demineralization or caries.

INTRODUCTION

For many decades, fluoride release from dental materials and uptake into tooth structure has been studied extensively. Results from numerous research studies have established that glass ionomer restoratives release significant amounts of fluoride and are effective in preventing recurrent decay and demineralization. Over the past decade, new materials with fluoride have been developed, including hybrid ionomers (also called resin-modified glass ionomers, or RMGICs), compomers (also called polyacid-modified resin composites), and fluoride-containing composite resin. Along with the many new materials available to dentists come questions regarding their fluoride release and ability to inhibit demineralization. Many of these materials are used by today's dental clinicians for permanent restorations or even bases and liners, providing protection to pulpal tissues. Dentists often may use these materials anticipating that they will release fluoride and provide protection from recurrent decay or remineralize decalcified tooth structure.

Introduced in the late 1980s, hybrid ionomers are similar to glass ionomers, but differ in that acidic and polymerizable polymers are present and set through both acid/base and polymerization reactions.[1] Advantages of hybrid ionomers over traditional glass ionomers include longer working times and controlled setting times (through light-curing), fast development of strength and lower sensitivity to environmental moisture changes. Hybrid ionomers can also be finished and polished immediately after setting with minimal deleterious effects on their properties. Various research studies have shown hybrid ionomers to have fluoride-releasing capabilities similar to traditional glass ionomer restoratives.[2,3,4,5,6] However, many of these studies have measured fluoride release from materials directly into aqueous solution. Few of these studies have attempted to correlate the measured fluoride release in an aqueous environment with the fluoride released into dentin. Such findings are of limited value, as they do not report the depth of fluoride uptake into tooth structure. In fact, studies on the efficacy of fluoride release from dental materials are innumerable, but the amount and depth of fluoride incorporation into dentin are not well-defined.[7] Furthermore, the concentration and depth of fluoride penetration from glass ionomer-based restoratives has not been adequately studied as a function of time for restorative materials in contact with dentin.

There are two main approaches for evaluating fluoride uptake into dentin. One approach is to quantify fluoride directly through chemical or physical methods such as ion or gas chromatography, ion-selective electrode measurements or electron microprobe analysis. While direct measurement of fluoride within tooth structure is an excellent method of evaluation, it does not demonstrate the cariostatic properties of the tooth. The other approach is to measure fluoride incorporation into dentin indirectly by assessing the inhibition of demineralization, acid resistance, or resistance to carious attack. Microradiography, polarized light microscopy, or hardness measurements may be employed for this assessment. The most widely used method to measure fluoride release directly is through the use of an ion-selective electrode. As a relatively easy, reproducible method of fluorine measurement with very high accuracy, ion-selective electrodes have been used extensively for comparing the fluoride release of different materials and to quantify the release in aqueous solution. Additionally, fluoride content in dentin can be assessed by dissolution or abrasion biopsy of the dentin and subsequent fluoride ion measurement. Despite the many advantages of ion-selective electrode measurement of fluoride, a major drawback to this method is that it does not accurately assess the interaction of a restorative material and the dentin, including fluoride diffusion depths. Also, the test specimen is destroyed in the process of fluoride analysis.

Of the studies utilizing direct measurement of fluoride release into dentin from restorative materials, many have utilized electron microprobe analysis. Electron microprobe studies of fluoridated amalgam are numerous,[8,9,10,11] as are similar research studies on fluoridated composites[3,12,13,14] and glass ionomer based materials.[3,15,16] With an extensive amount of research on fluoride release from these restorative materials, the glass ionomer based materials have shown the greatest propensity toward fluoride release, both into dentin as well as in aqueous solution.[2,6,17,18,19] Furthermore, numerous studies have shown the high glass ionomer fluoride release indirectly through the use of a demineralization sequence followed by either microradiography[4,7] or polarized light microscopy.[20,21] However, no studies to date have been able to accurately correlate fluoride concentration within tooth structure to a measurable resistance to demineralization.

The primary objective of the proposed research was to use electron microprobe analysis to measure the amount of fluoride uptake and the depth of penetration into dentin from a resin-modified glass ionomer restorative material after different time intervals. Additionally, each

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electron microprobe specimen was subjected to a demineralization challenge and the microhardness and Young's moduli measured in the dentin at specified distances from the tooth-restorative interface in an attempt to correlate fluoride concentration within dentin to demineralization resistance.

METHODS AND MATERIALS

Twenty intact human molars with no carious lesions, cracks, or visible enamel defects in the area to be restored were selected from a collection of recently extracted teeth. The teeth were disinfected and preserved in 0.2% sodium azide solution prior to specimen preparation. Using a high-speed handpiece and #330 carbide bur with cold water spray, cylindrical preparations measuring approximately 2 mm in depth and 3 mm in diameter were drilled within the mid-buccal of each tooth, with the apical extent of the preparation 1-2 mm above the cemento-enamel junction. All cavities were prepared two days prior to restoring and stored at room temperature. One day prior to restoring, each specimen was coated with 2 layers of nail varnish and allowed to dry at room temperature in order to seal the tooth from leakage. At the time of restoring each specimen, a 20% polyacrylic acid cavity conditioner (GC cavity conditioner, GC America, Alsip, IL) was applied for 10 seconds, rinsed and dried until the dentin was just moist. Capsules were triturated using an ESPE Capmix (3M ESPE, Minneapolis, MN) for 10 seconds. The cavity preparations were restored using Fuji II LC encapsulated hybrid ionomer (Lot 0106223, GC America, Alsip, IL) following manufacturer recommendations. Cavosurface margins were leveled prior to light curing using a carving instrument. Each restoration was light-cured using an Optilux 501 halogen light-curing unit (Kerr Co., Orange, CA) for 20 seconds. The restorations were neither finished nor polished. For all restorations, shade A2 was used. The teeth were randomly divided into groups of 5 specimens, with time intervals of 1, 15, and 30 days allotted for restoration maturation before sectioning and measuring fluoride uptake. Within 5 minutes after restoration placement, the teeth were wrapped in paper towel saturated with distilled water and placed in a sealed, 100% humidity environment. In addition to the Fuji II LC-restored groups, a set of 5 control specimens was made, containing Valiant Ph.D. (Ivoclar Vivadent, Inc., Amherst, NY), a non-fluoridated amalgam. Valiant Ph.D. capsules were triturated using an ESPE Capmix and condensed into the Class V preparations following

manufacturer guidelines and the cavosurface margins leveled to eliminate overhangs. The control specimens were placed in a 100% humidity environment and were matured for 30 days.

At the conclusion of the 1, 15, and 30 day maturation times, the amalgam control and Fuji II LC specimens were properly oriented and mounted in Koldmount fast-setting acrylic resin (IDP/Vernon-Benshoff Co., Albany, NY) and sectioned to the approximate mid-point of the 3 mm diameter restorations using a 240 grit Isomet slow speed diamond saw (Buhler Ltd., Lake Bluff, IL). Each specimen was then hand-polished wet using 400, 600, 1200 grit and 8 μm and 3 μm SiC polishing papers (Mager Scientific, Dexter, MI). A 1 μm diamond paste (Mager Scientific, Dexter, MI) was used for final hand-polishing. The non-fluoridated amalgam control specimens were sectioned 30 days after restoring, mounted and polished like the experimental specimens. All specimens were ultrasonically cleaned in distilled water for 5 minutes to remove debris and then dried overnight at 37°C for approximately 12 hours. Figures 1 and 2 show representative sectioned specimens ready for electron microprobe analysis.

Figure 1. Sectioned experimental specimen mounted in Koldmount acrylic.

Figure 2. Magnified photo of the specimen in Figure 1, demonstrating the Class V Fuji II LC cross-section into dentin.

Prior to microprobe analysis, the specimens were coated with a thin film of carbon under a vacuum. The specimens were subjected to electron microprobe analysis using a Cameca SX 100 Electron Probe Microanalyzer (Cameca Instruments, Inc., Trumbull, CT) to measure fluoride content within dentin at the tooth-restorative interface. For each specimen, a linear analysis for fluorine, calcium, phosphorus, and silicon was done at the approximate center of the axial wall interface (dentin-restorative interface), with analysis points located 2 μm apart. The line was oriented perpendicular to the tooth-restorative interface. One linear analysis per tooth

was performed. Microprobe settings included 10 kV, 10 nA, a 1 μm beam focus, a 2.5 μm beam raster, and 60 second analysis time per point, with a 30 second background level analysis (subtracted from the overall raw counts). A conversion of fluorine raw counts to weight percent was done using a fluorapatite standard with 3.67 wt. % fluorine. A point analysis on the fluorapatite standard provided a peak count reference for fluorine at 3.67 wt. %, from which the experimental specimen fluorine peak counts were compared. The average fluorine detection limit during experimental analysis was approximately 450-500 ppm.

Subsequent to electron microprobe analysis, each specimen was re-polished using 1200 grit, 8 μm and 3 μm SiC paper, followed by 1μm diamond polishing paste. Thereafter, each specimen was ultrasonically cleaned in distilled water for 5 minutes and air dried. Demineralization of each specimen from the three maturation groups (1, 15, and 30 days) was performed ten to twelve hours following polishing. For the 30 day control group, specimens were first subjected to nanoindentation (designated the 30 day undemineralized control group), then demineralized similarly to the three maturation groups. Each specimen was immersed in 40 mL of a 0.1M acetic acid buffer solution (sodium acetate/acetic acid), pH 4.5, for exactly 1 minute. After the demineralization sequence, each specimen was rinsed immediately with distilled water and again ultrasonically cleaned for 5 minutes. Specimens were allowed to air-dry at room temperature following this sequence and then subjected to nanohardness measurements using a Nanoindenter XP automated microhardness instrument (MTS Systems Co, Eden Prairie, MN). Ten measurements per specimen were taken in the dentin along a 100 μm line perpendicular to the axial wall. Indents were measured every 10 μm starting 10 μm into dentin from the tooth-restorative interface. A three-sided diamond pyramidal indent head, with a maximum force of 40 mN was used for each indentation point. Each indent consisted of fifteen seconds of ramped loading, followed by a 30 second hold time at maximum force, and 15 seconds of ramped unloading. Nanohardness and Young's modulus were calculated automatically for each indent. No calibrations of the Nanoindenter XP instrument were necessary between specimen groups.

Statistical analysis of the fluorine levels measured from electron microprobe analysis was conducted using a one-way ANOVA, comparing specimens at each 2 μm distance interval. Levene's test for homogeneity of variance was used to evaluate whether the assumption of constant variance of the residuals from each set of specimens was violated. The variance of

residuals was considered constant at a significance of p<0.05. At the distances (6, 12, and 18 μm from the interface) where the assumption was violated, Welch's test was used as a robust correction to the ANOVA.

RESULTS

Fluoride Uptake Into Dentin

Determination of the tooth-restorative interface for each specimen using an electron microprobe was done through the analysis of calcium and phosphorus content. Starting within the restorative material (or a gap between the tooth and restoration), calcium and phosphorus significantly increased within a narrow distance as analysis points enter dentin, while silicon (present in high amounts in the restorative material but not in tooth) decreased significantly. Silicon analysis also demonstrated the absence of any smearing of the fluorine-rich particles from the glass ionomer onto the dentin surface. The analysis point at which calcium and phosphorus almost reach their baseline tooth levels, silicon decreases to nearly zero, and fluorine spikes up to a peak was the beginning point of analyzing locations in dentin. This point was designated as being 2 μm into dentin from the restorative-tooth interface.

Table 1 shows the average values and standard deviations for fluorine, phosphorus, and calcium throughout the electron microprobe linear analysis. The data clearly shows a significant increase in phosphorus and calcium weight percentage from the designated "Interface" to the 2 μm distance. Beyond the initial 2 μm, phosphorus and calcium content stay fairly constant. It was believed that the 20% polyacrylic acid cavity conditioner, applied prior to restoring each cavity restored with Fuji II LC, could partially account for the slight reduction in calcium and phosphorus near the interface. However, this reduction in interfacial calcium and phosphorus was most likely due to the resolution of the electron microprobe. If the tooth-restorative interface is not perfectly defined, as the microprobe beam travels over the interface, there may not be a clear distinction from one analysis point to another, resulting in a broader spike in calcium and phosphorus content, which may span a few micrometers. Indeed, in the 30 day control specimens, which were restored with Valiant Ph.D. non-fluoridated amalgam and had no cavity conditioner applied, a local interfacial reduction in calcium and phosphorus was encountered, similar to the 1, 15, and 30 day matured Fuji II LC specimens.

Table 1. Electron Microprobe Analysis F, P and Ca Averages.

Distance from interface,μm	1 Day			15 Days			30 Days			30 Day Control		
	ppm	Weight %		ppm	Weight %		ppm	Weight %		ppm	Weight %	
	F	P	Ca	F	P	Ca	F	P	Ca	F	P	Ca
-6		0.51	0.85		0.80	1.58		1.17	3.15		0.09	6.09
-4		0.78	1.37		0.95	2.78		1.66	4.25		0.24	9.84
-2		1.20	2.22		1.39	5.23		2.79	8.02		0.91	12.85
Interface		**1.65**	**4.92**		**3.98**	**10.75**		**6.56**	**17.19**		**3.14**	**14.69**
2	3752 ± 4880	11.19	23.90	5670 ± 1974	10.13	23.68	5618 ± 1537	13.14	27.85	286 ± 437	10.24	26.74
4	1750 ± 1193	13.80	27.46	5032 ± 1922	12.37	29.19	5288 ± 1558	14.34	30.10	84 ± 188	13.78	31.29
6	762 ± 611	14.73	29.41	4866 ± 806	14.17	32.67	3670 ± 1671	15.03	31.07	40 ± 89	14.61	32.53
8	616 ± 384	14.95	30.45	3848 ± 955	14.66	32.34	2502 ± 380	14.98	31.46	36 ± 80	15.13	33.26
10	320 ± 577	15.10	29.85	3318 ± 824	14.35	32.58	1930 ± 1099	14.38	32.15	160 ± 211	15.34	33.07
12	328 ± 458	14.21	30.27	2440 ± 1263	14.59	32.16	2172 ± 953	14.81	31.53	60 ± 82	15.58	32.80
14	440 ± 461	15.62	29.32	1630 ± 1190	14.67	32.01	1836 ± 897	14.52	31.77	46 ± 64	15.66	32.64
16	298 ± 487	13.52	30.08	808 ± 589	14.27	32.00	1306 ± 571	14.95	30.87	154 ± 312	15.67	32.80
18	324 ± 444	13.71	29.81	734 ± 781	13.36	31.46	1010 ± 623	14.65	31.79	312 ± 282	15.82	32.51
20	152 ± 200	14.31	30.04	354 ± 407	13.91	31.22	1204 ± 316	15.42	30.64	94 ± 210	15.81	32.78
22	102 ± 228	14.28	30.48	686 ± 575	13.49	30.14	1072 ± 540	15.18	30.98	130 ± 274	15.77	32.52
24	174 ± 238	14.93	28.96	758 ± 758	12.97	30.03	766 ± 515	15.03	31.19	188 ± 420	15.41	32.18
26	334 ± 436	13.94	29.84	578 ± 1097	13.64	30.27	496 ± 460	15.11	31.15	154 ± 312	15.48	32.76
28	226 ± 457	14.00	29.63	418 ± 653	13.60	30.82	820 ± 609	14.79	31.34	178 ± 348	15.67	32.98
30	136 ± 304	13.20	30.11	506 ± 476	14.05	32.24	946 ± 1287	15.16	31.17	246 ± 363	15.67	32.82
32	358 ± 373	13.50	29.85	1024 ± 1341	13.09	30.22	1094 ± 1009	15.19	30.70	104 ± 97	15.36	32.78
34				658 ± 641	12.77	32.71	254 ± 275	14.28	31.30	335 ± 670	15.69	32.66
36				336 ± 389	13.91	31.71	406 ± 370	14.79	30.77	93 ± 185	15.62	32.73
38				96 ± 143	14.25	31.21	630 ± 417	15.41	30.83	178 ± 348	15.68	32.37

Figure 3 shows the electron microprobe analysis results for the three experimental groups and the control group. Fluorine weight percent is displayed as a function of distance from the tooth-restorative interface. The control group demonstrated negligible fluorine content throughout the dentin, while the 1 day group showed fluorine above baseline levels only within the first few micrometers. An interfacial fluorine level of 3,800 ppm is seen for the 1 day specimens, which drops to below the detection limit within 10 μm. However, the 15 and 30 day groups had significant average fluorine penetration up to about 20 μm, with interfacial fluorine levels almost identical, at around 5,600 ppm.
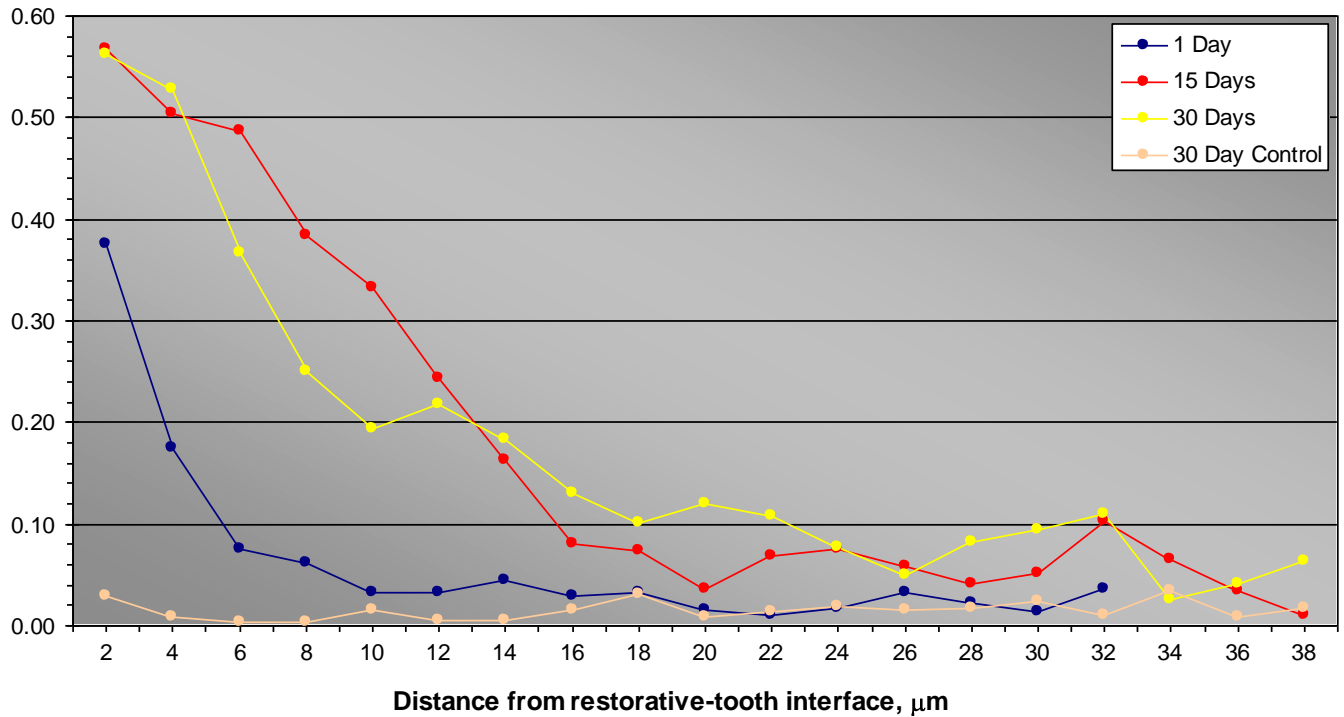
Weight % Fluorine

Figure 3. Fluorine concentration within dentin as a function of specimen maturation time and distance from the tooth-restorative interface.

The data from Figure 3 is presented in Figures 4, 5, 6, and 7, with fluorine, calcium, and phosphorus content displayed individually for each group of specimens. Additionally, standard deviation error bars are displayed, along with the fluorine detection limits, which were calculated by the electron microprobe software for each analysis point. Average fluorine ppm values (shown in Table 1 and Figures 4-7) lower than the detection limit signify fluorine levels that were below that reliably measured using the electron microprobe. However, the actual fluorine concentration at specific distances may still be well-above normal dentin levels but just below the approximate 450-500 ppm detection limits. Additionally, for individual specimens, fluorine concentrations at specific distances may have been above the detection limit, but the average fluorine concentration for the group of specimens was below the limit. Due to the rapid decline in fluorine content to below the detection limit with the 1 day group specimens, electron microprobe analysis was only conducted to a distance of 32 μm from the tooth-restorative

interface. For all other groups and specimens, analysis was conducted to at least 38 μm from the tooth-restorative interface.

Wt. % Phosphorus, Calcium

ppm Fluorine
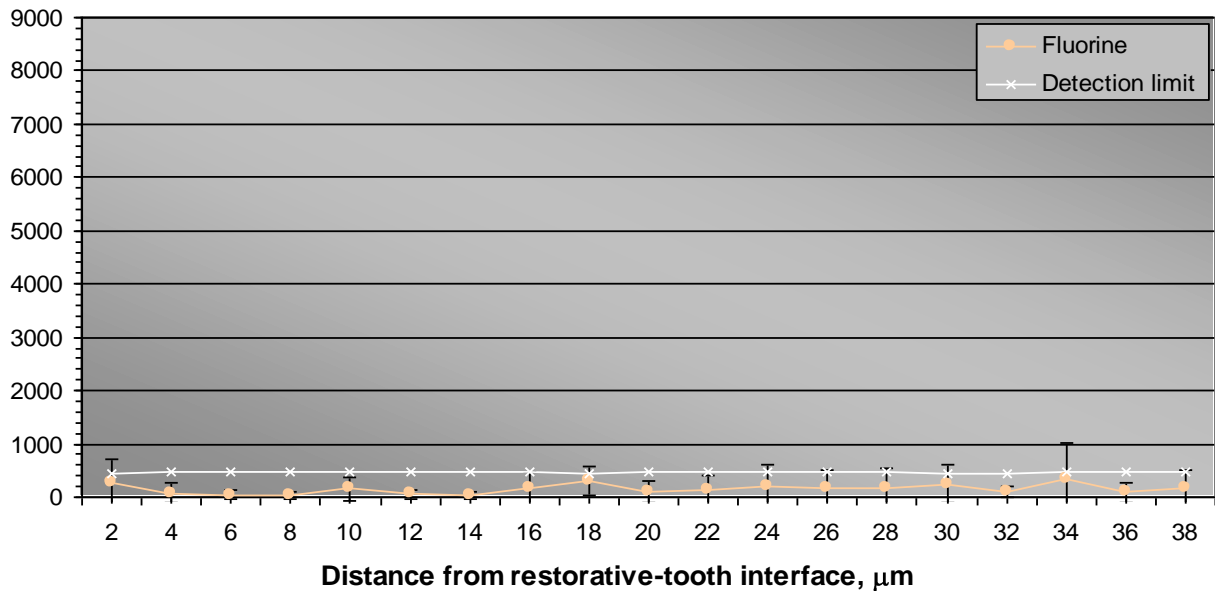

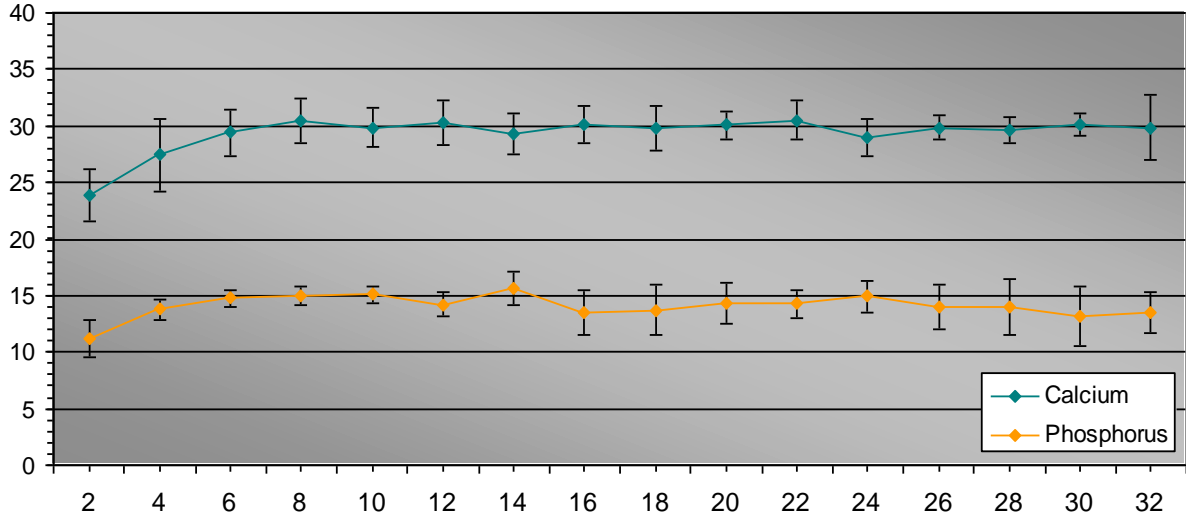Distance from restorative-tooth interface, μm
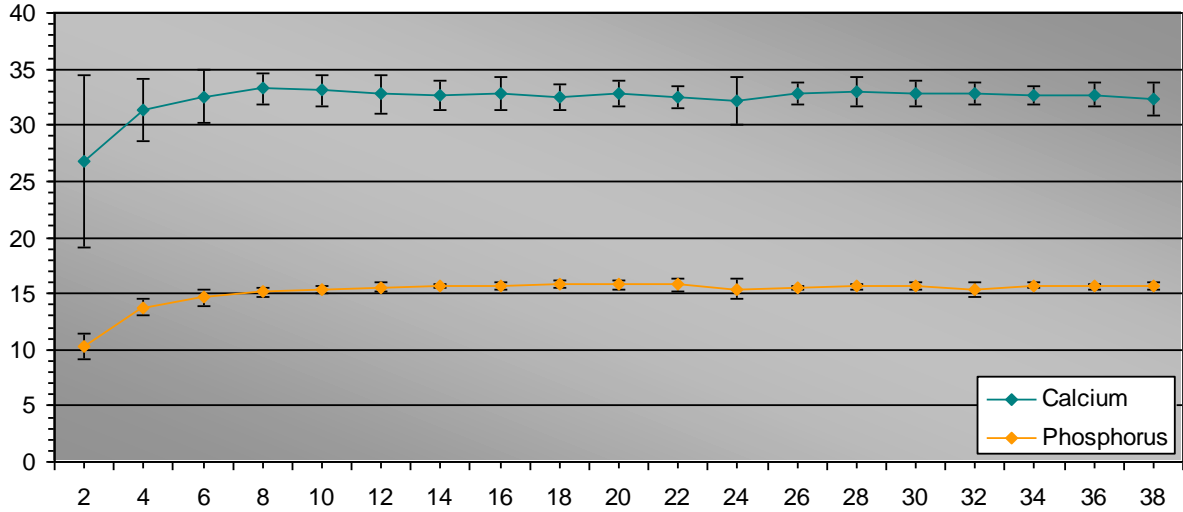
Figure 4. 30 day (Valiant Ph.D.) control specimen average calcium, phosphorus, and fluorine concentrations within dentin as a function of distance from the tooth-restorative interface.

Figure 5. 1 day specimen average calcium, phosphorus, and fluorine concentrations within dentin as a function of distance from the tooth-restorative interface.

Wt. % Phosphorus, Calcium

ppm Fluorine

Figure 6. 15 day specimen average calcium, phosphorus, and fluorine concentrations within dentin as a function of distance from the tooth-restorative interface.

Wt. % Phosphorus, Calcium

ppm Fluorine

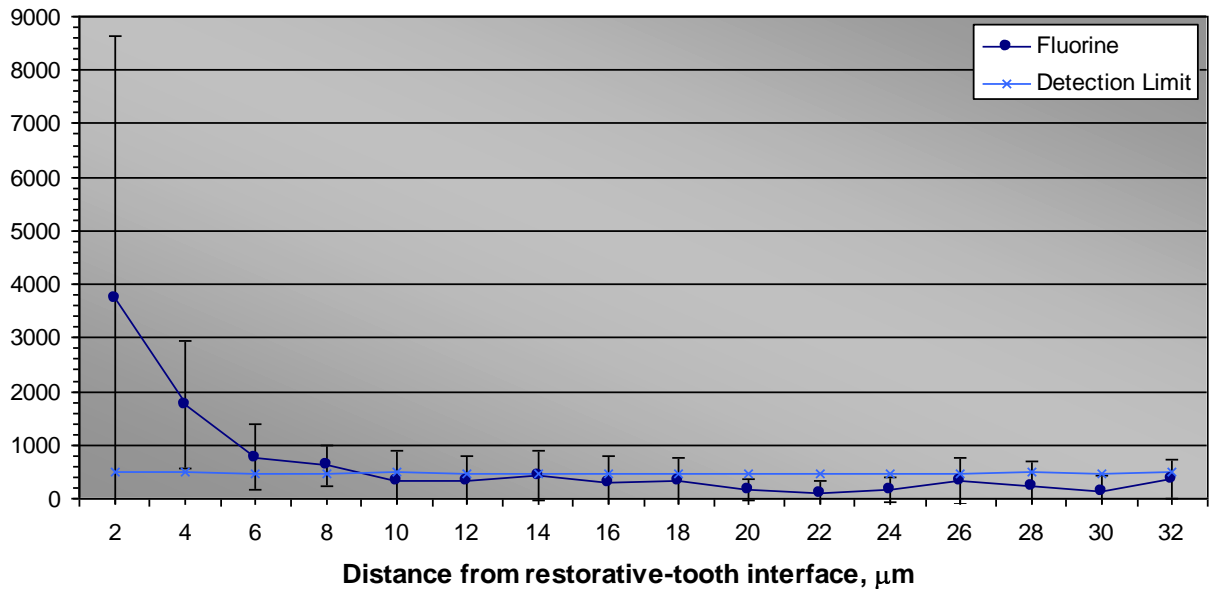
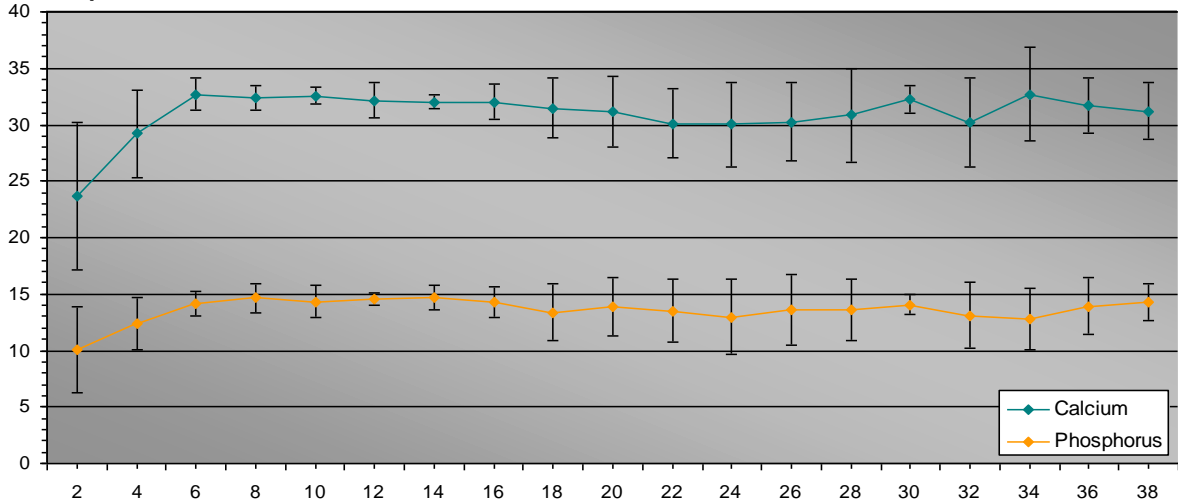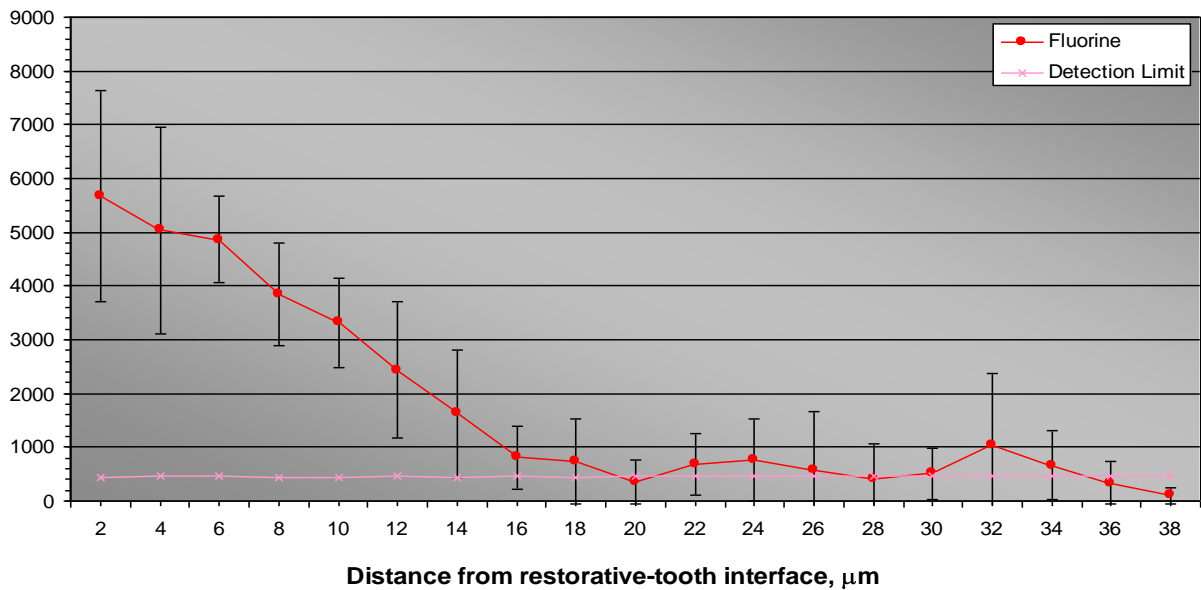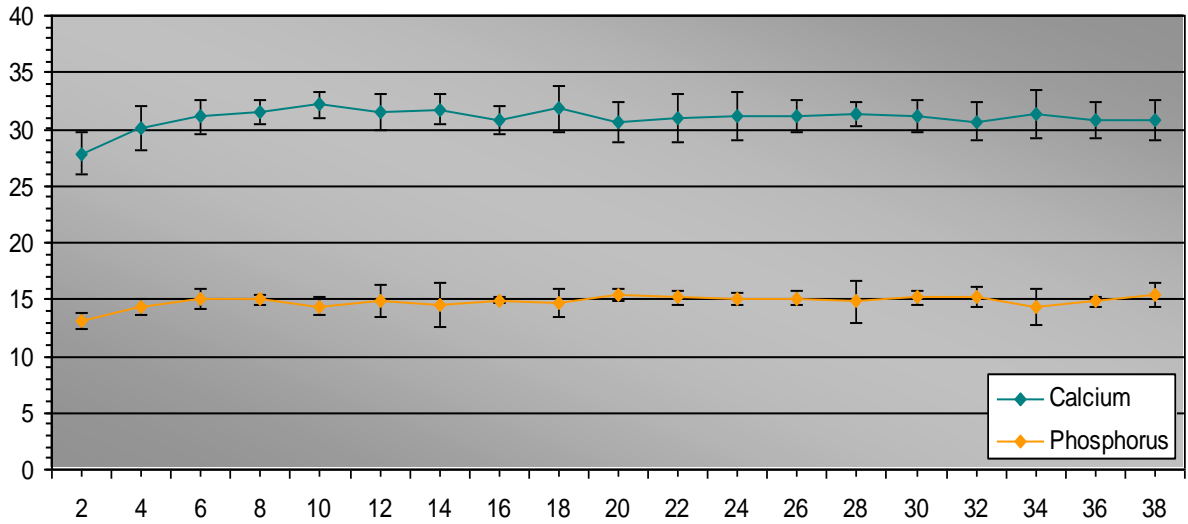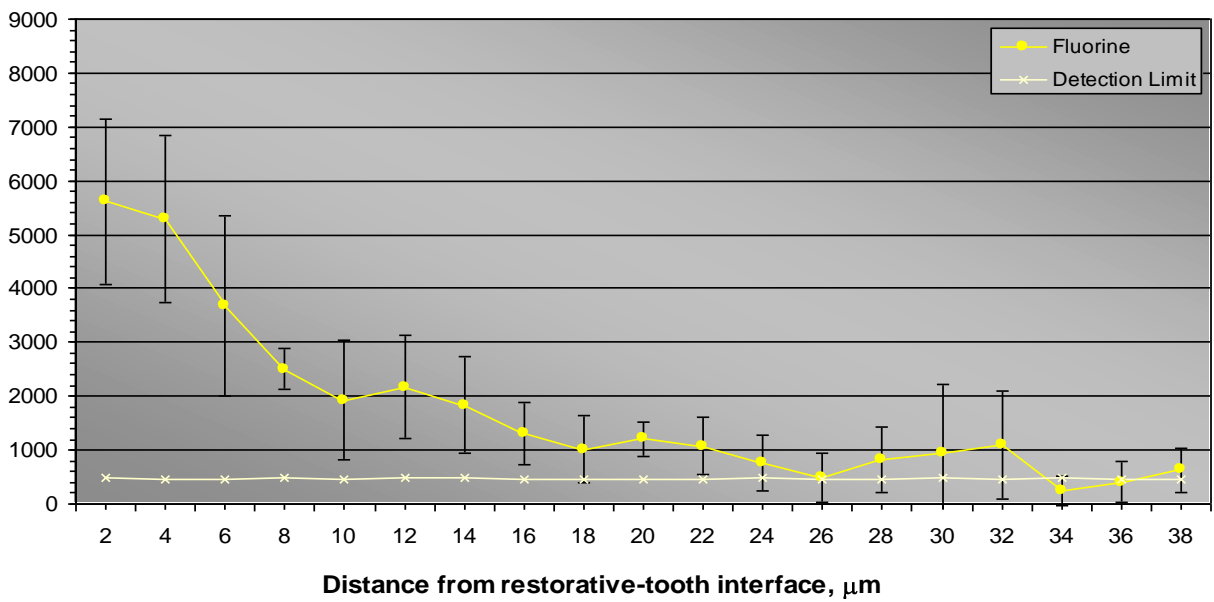
Distance from restorative-tooth interface, μm

Figure 7. 30 day specimen average calcium, phosphorus, and fluorine concentrations within dentin as a function of distance from the tooth-restorative interface.

Table 2 shows the results of the one-way analysis of variance at each 2 μm interval from the restorative-tooth interface. There were no significant differences seen between the 30 day control and 1 day specimens. However, with the 15 day specimens, there was significantly more fluoride detected in dentin within the first 12 μm compared to the controls. Likewise, for the 30 day specimens, there was more fluoride detected within the first 16 μm. Similar differences were seen between the 1 day specimens and the 15 and 30 day specimens. Few differences in dentin fluoride content were detected between the 15 and 30 day specimens and there were no significant differences between any of the specimens noted beyond 24 μm. Any differences with a p-value above 0.05 were considered statistically insignificant.

Table 2. Statistically Significant Differences in Fluoride as a Function of Time and Distance.

Maturation Time	Distance from the restorative-tooth interface, μm											
	2	4	6	8	10	12	14	16	18	20	22	24
30 Day Control < 1 Day												
30 Day Control < 15 Days	✓	✓	✓	✓	✓	✓						
30 Day Control < 30 Days	✓	✓	✓	✓	✓	✓	✓	✓				
1 Day < 15 Days		✓	✓	✓	✓	✓						
1 Day < 30 Days		✓	✓	✓	✓	✓		✓		✓	✓	
15 Days < 30 Days										✓		
30 Days < 15 Days				✓								
df1,df2	3,16	3,16	3,6.82	3,16	3,16	3,6.85	3,16	3,16	3,8.32	3,16	3,16	3,16
F-value	4.04	17.62	58.78	49.38	16.62	10.61	5.49	5.28	1.80	15.13	5.85	2.10
P-value	0.05	0.01	0.01	0.01	0.01	0.01	0.01	0.05	0.05	0.01	0.01	0.05

Demineralization Challenge and Nanoindentation Measurements

Figures 8 and 9 show a representative 15 day specimen under SEM subsequent to mounting and sectioning, and after the demineralization sequence using 0.1M buffered acetic acid. The primary noticeable difference between the two photos is the opening and widening of the dentin tubules. Furthermore, under light microscopy used with the Nanoindenter XP instrument, the surface of the demineralized specimen appeared rougher than the undeminerlized counterpart. Avoiding dentin tubules during the indentation procedures was attempted, but complicated due to the enlarging of the tubules. Figure 10 demonstrates average nanohardness at
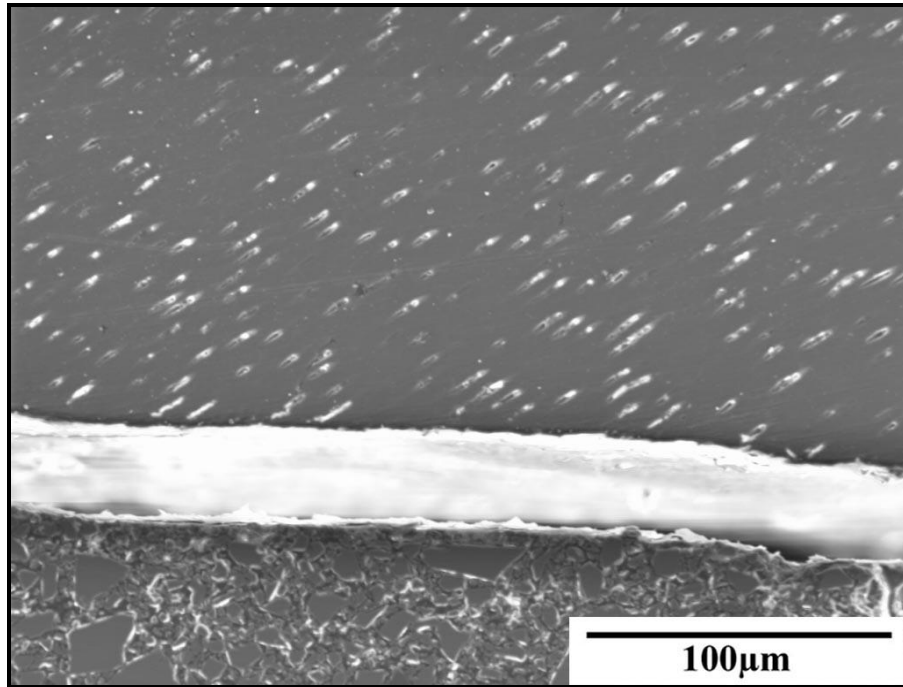
FIGURE 8. SEM photo of a 15 day matured Fuji II LC specimen. The dentin (above) demonstrates small tubules throughout the surface. The restorative (below) demonstrates the multiple phases of the material. The white band shows the interface gap formation likely due to dehydration of the dentin and restorative material.
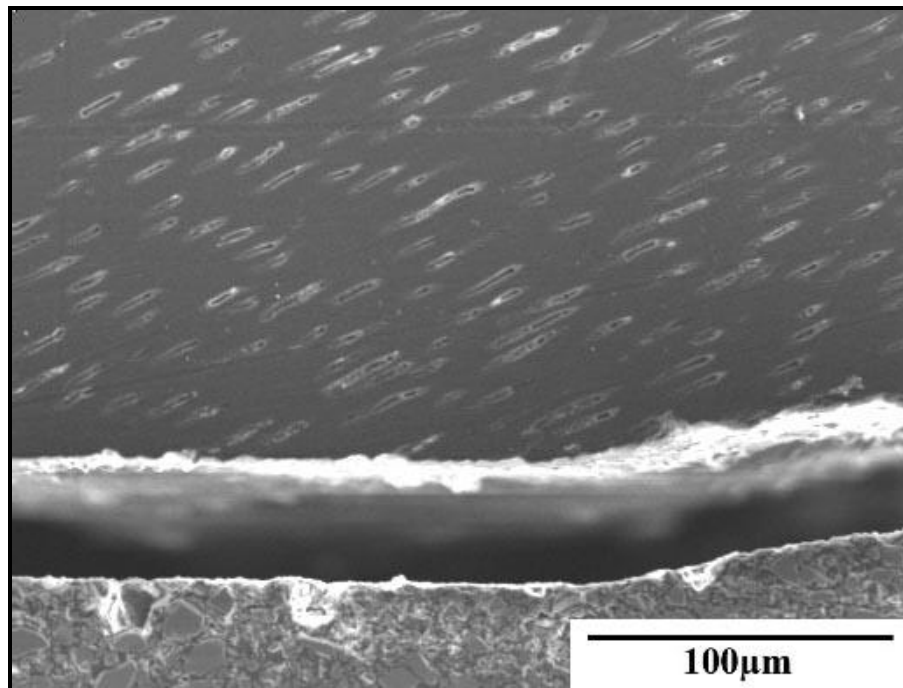
FIGURE 9. SEM photo of the same 15 day matured Fuji II LC specimen after 1 minute of exposure to 0.1M buffered acetic acid. The dentinal tubules appear larger and unoccluded.

Hardness at 40mN, MPa

Distance from restorative-tooth interface, μm

Legend: 1 Day, 15 Days, 30 Days, 30 Day Demineralized Control, 30 Day Undemineralized Control

Figure 10. Average nanohardness of demineralized dentin measured at the tooth-restorative interface.

Young's modulus, GPa

Distance from restorative-tooth interface, μm

Legend: 1 Day, 15 Days, 30 Days, 30 Day Demineralized Control, 30 Day Undemineralized Control

Figure 11. Average Young's moduli of demineralized dentin measured at the tooth-restorative interface.

a maximum load of 40 mN over a span of 100 μm from the tooth-restorative interface, while Figure 11 shows the average Young's moduli determined from each set of specimen indents.

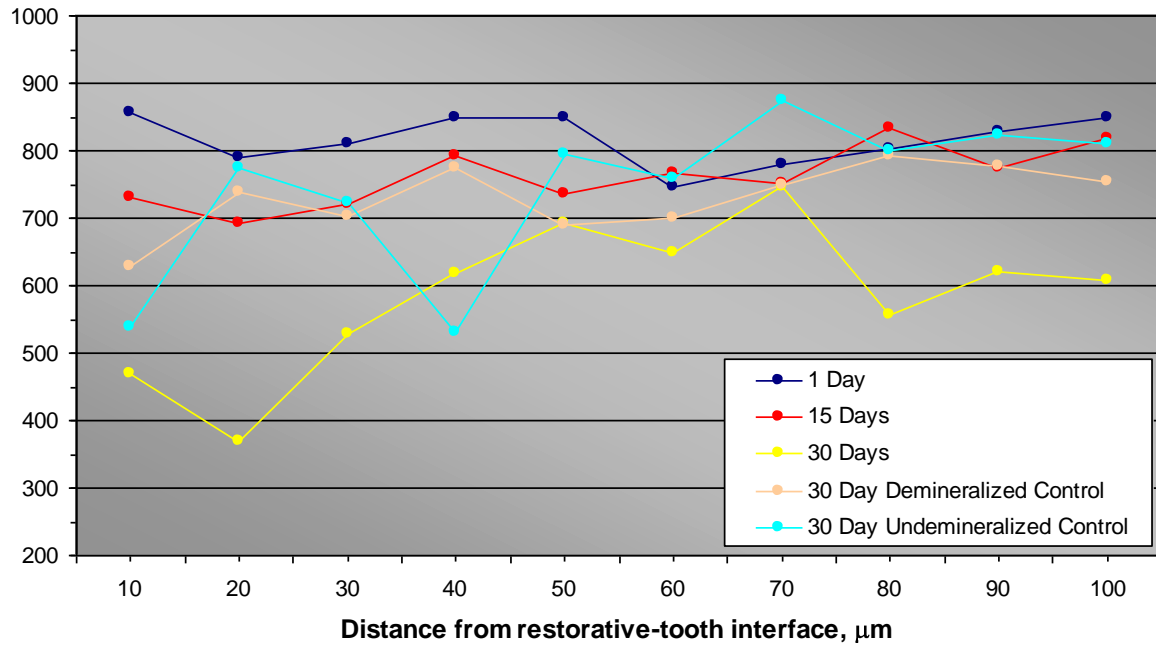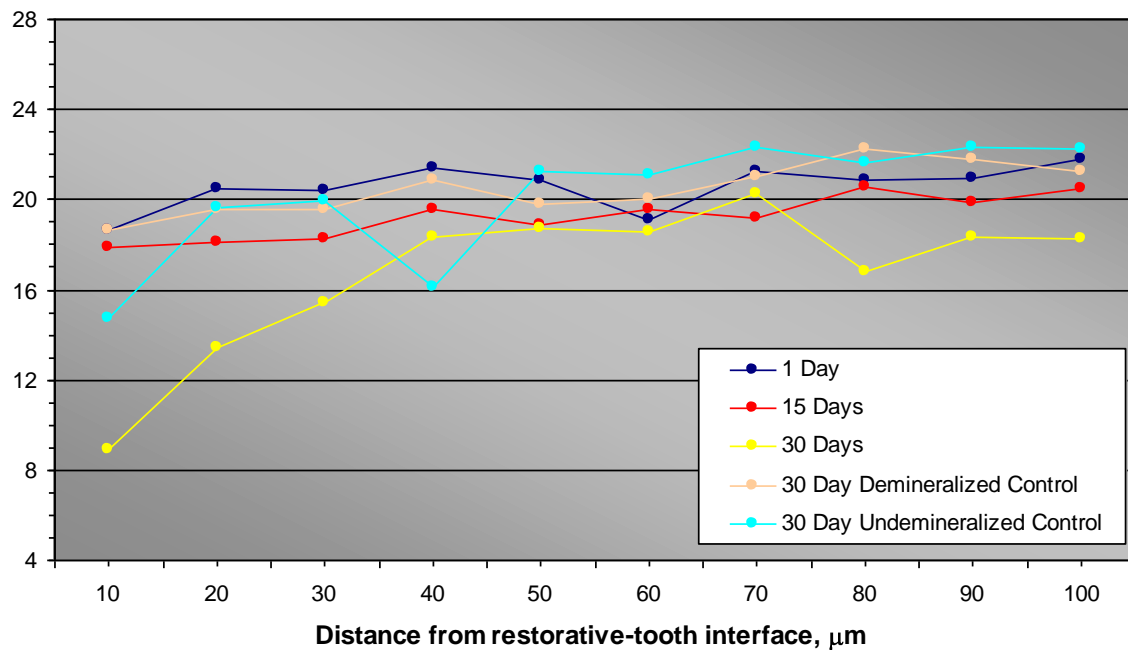Table 3 shows the mean values for nanohardness and Young's moduli displayed in Figures 10 and 11. Additionally, standard deviations are shown for all specimens. Indents were made on the same specimens used for electron microprobe analysis. With the exception of the 30 day specimens, there are no apparent differences between any of the groups compared at a specific distance nor any differences within a group as a function of distance from the tooth-restorative interface. In general, for the 30 day specimens, 30 day demineralized control specimens, and 30 day undemineralized control specimens, the first indentation (at 10 μm from the interface) produced lower hardness and moduli values as well as the largest variation, as demonstrated by the high standard deviations.

Table 3. Nanohardness and Young's Modulus Averages and Standard Deviations.

Distance from interface, μm	1 Day		15 Days		30 Days		30 Day Demineralized Control		30 Day Undemineralized Control	
	Hardness	Young's Modulus	Hardness	Young's Modulus	Hardness	Young's Modulus	Hardness	Young's Modulus	Hardness	Young's Modulus
10	855.3 ± 76.4	18.63 ± 2.15	731.0 ± 81.5	17.90 ± 2.21	470.3 ± 392.1	8.92 ± 9.04	628.0 ± 334.1	18.63 ± 4.17	539.3 ± 173.6	14.77 ± 3.80
20	789.5 ± 108.1	20.52 ± 3.59	691.3 ± 83.8	18.10 ± 1.49	368.7 ± 249.6	13.45 ± 4.78	738.0 ± 81.4	19.60 ± 2.47	773.2 ± 85.3	19.62 ± 2.01
30	810.6 ± 118.8	20.44 ± 2.44	720.8 ± 134.4	18.24 ± 2.92	528.8 ± 282.7	15.45 ± 3.54	703.8 ± 85.1	19.60 ± 1.86	724.0 ± 84.4	19.93 ± 1.29
40	849.0 ± 122.6	21.40 ± 0.88	791.8 ± 110.7	19.56 ± 2.55	618.4 ± 192.9	18.32 ± 3.54	774.4 ± 233.8	20.90 ± 3.18	530.0 ± 329.6	16.11 ± 6.14
50	847.8 ± 96.8	20.85 ± 1.42	735.6 ± 76.9	18.86 ± 2.03	691.8 ± 172.1	18.75 ± 3.75	689.4 ± 137.8	19.80 ± 2.27	793.8 ± 66.4	21.24 ± 1.40
60	747.4 ± 72.6	19.12 ± 1.04	765.4 ± 134.5	19.58 ± 2.49	650.0 ± 190.9	18.56 ± 3.26	699.0 ± 64.9	20.00 ± 1.34	760.3 ± 47.4	21.08 ± 0.69
70	779.8 ± 27.1	21.24 ± 1.11	750.4 ± 109.5	19.22 ± 2.25	747.0 ± 82.4	20.27 ± 3.75	747.6 ± 120.6	21.04 ± 2.35	875.4 ± 231.2	22.32 ± 2.68
80	803.6 ± 101.9	20.84 ± 2.44	833.2 ± 164.2	20.54 ± 3.26	556.4 ± 253.7	16.84 ± 3.70	792.8 ± 249.8	22.24 ± 3.60	799.2 ± 50.6	21.64 ± 0.64
90	827.6 ± 109.8	20.94 ± 1.88	774.0 ± 121.8	19.84 ± 2.28	621.0 ± 158.9	18.34 ± 2.49	776.0 ± 110.3	21.76 ± 1.90	823.4 ± 56.4	22.36 ± 1.15
100	849.0 ± 132.4	21.78 ± 2.41	817.4 ± 107.1	20.50 ± 1.87	608.4 ± 126.4	18.28 ± 2.43	753.6 ± 124.8	21.28 ± 2.06	809.4 ± 83.3	22.24 ± 0.56

DISCUSSION

The results of the electron microprobe analysis of specimens confirm that fluoride is released into dentin from Fuji II LC restorations in detectable amounts and diffuses over time. Other studies of fluoride uptake into dentin have shown similar maximum fluoride levels,

penetration depths and depths of demineralization inhibition. For instance, Mukai et al. (1993) reported on the fluoride release from the glass ionomer Vitrabond, with maximum fluoride levels reaching about 7,000 ppm near the restorative interface.[19] Han et al. (2001) demonstrated that most of the fluoride from Fuji Liner LC was released after just the first 7 days.[21] Other studies measuring the diffusion distance of fluoride have confirmed that over periods of time 1 month or less, fluoride may be expected to penetrate up to 100 μm into dentin. In a study by Yamamoto et al. (2000), after 1 month, the maximum fluoride concentrations at the axial wall of Class V restorations restored with Fuji II LC averaged 5,600 ppm, with penetration depths of around 38 μm.[3] In a follow-up study comparing in vivo and in vitro specimens, Yamamoto et al. (2001) showed maximum in vitro fluoride uptake to be 6,700 ppm after 1 month, with an average measurable penetration depth of 79 μm.[13] In vivo fluoride levels were similar, with penetration depth reaching 40 μm on average. These numbers coincide with those seen in this study. After maturation in 100% humidity, the 30 day specimens in this study showed an average interface fluoride concentration of 5,600 ppm, with detectable fluoride levels (above about 500 ppm) reaching over 30 μm from the interface. However, compared to the 30 day control specimens, there was only statistical significance in fluoride levels to 16 μm from the interface. Such statistical analyses were not conducted in any other similar electron microprobe studies of fluoride release.

There are three mechanisms that fluoride is known to act on tooth structure: 1) prevention of demineralization, 2) enhancement of remineralization, and 3) inhibition of cariogenic bacteria enzymes.[22] While measuring the absolute fluoride levels within dentin may be important in assessing the ability to resist demineralization, measurement of fluoride penetration may be important primarily because of the effects of fluoride on enhancing remineralization. Some have suggested that even at high fluoride levels such as 1000 ppm in enamel, there is no measurable benefit against demineralization from acid.[23] It follows that even if fluoride penetration at lower amounts may not provide deeper resistance to acid attack, it can allow a greater capacity for remineralization after such an attack.

In addition to fluoride levels and penetration into dentin, this study investigated changes over time. The results clearly demonstrated higher fluoride concentrations within dentin after specimen maturation time beyond 1 day. However, no significant fluoride differences were seen between the 1 day, 15 day, and 30 day specimens directly at the interface. Furthermore,

significant differences in fluoride content between 15 and 30 day specimens were minimal throughout the penetration depths. These results are supported by other time-dependent studies. For instance, Yamamoto et al. (1996) reported on the fluoride release and diffusion patterns of a conventional glass ionomer.[16] Results showed that after 1, 3, and 6 months of maturation, fluoride penetration increased with time, but the surface concentrations were not statistically different over time. Shibatani et al. (1989) measured fluoride release from a fluoridated resin using electron microprobe analysis.[12] The study demonstrated that over time, fluoride penetration into dentin increased slowly up to 180 days of maturation, while surface fluorine content was maintained fairly well within the superficial 50 μm.

For this study, two explanations may describe the similar fluoride concentration curves for the 15 and 30 day specimen groups seen in Figure 3. First, it is possible that the majority of fluoride release and diffusion from the glass ionomer into the dentin occurred within the first few days. Thereafter, there was minimal or no significant fluoride release, but diffusion within dentin may have been very slow beyond the first 20-30 μm from the interface. The other possibility is that, beyond 15 days of maturation, small amounts of fluorine were released into the dentin concurrently as the initially released high level of fluorine slowly diffused deeper into dentin. The diffusion may have caused deeper fluorine penetration, but below the 500 ppm detection limit it was not noticeable with the electron microprobe instrumentation and settings. The best description of the curves may include both of the above explanations. As previously pointed out, the fluoride release from glass ionomers may initially be high and can be attributed to surface dissolution and initial setting. Over time, bulk diffusion within the restoration is the primary mechanism for fluoride release. This mechanism is very slow, and results in significantly less fluoride release into dentin. Diffusion within dentin is also a slow process— one that may be even slower in vitro compared to in vivo. In vivo setting and maturation of glass ionomers and resin-modified glass ionomers may theoretically have an impact on fluoride release and diffusion; the hydration of teeth in vivo and the presence of hydrostatic pressure in dentin tubules may create an aqueous medium for much faster fluoride ion transport. Although the results of this study are encouraging in proving fluoride release and penetration from a resin-modified glass ionomer, additional studies need to be conducted to demonstrate the long-term diffusion patterns of fluoride from glass ionomer-based materials, over periods of 6 months or longer.

An explanation of the nanoindentation testing procedure and determination of the mechanical properties from each indent is important when considering the results. For each specimen, nanohardness testing was performed automatically through the Nanoindenter XP instrument. The Nanoindenter XP uses an optical microscope to visualize the specimens to be indented. Therefore, there was a relative lack of clarity of the surface compared to SEM analysis. An etched surface, with rougher morphology and larger dentin tubule openings presented a problem in finding relatively smooth surfaces to accurately measure nanohardness. This dilemma was the primary reason for choosing a 1 minute demineralization time for each specimen. Lengthier demineralization of the surfaces resulted in greater roughness, which made nanoindentation far less predictable and consistent. In the indentation testing procedure, the tooth-restorative interface was identified for each specimen through an optical microscope and a section with fairly smooth surface anatomy chosen to begin the series of 10 indents. With a nanoindenter load of 40 mN, each indent penetrated around 1.5-2.5 μm into the dentin surface, but no further. Thus, primarily the surface characteristics were measured, and not the bulk properties of the dentin. During the indent sequence, hardness and Young's modulus for each indent was automatically calculated. No calibration was made for the Nanoindenter XP instrument between testing of different specimens or specimen groups.

Hardness is a measure of the peak indentation load divided by the projected area of the indentation, described in equation 1, below. Equation 2 describes the elastic modulus (Young's modulus) of a material. This equation is based on the assumption that Young's modulus is equivalent to a value termed the "reduced modulus," which also accounts for the stiffness of the indenter tip and requires Poisson's ratio values for both the specimen tested and the indenter.

Equation 1.   H = Hardness
                  P_{max} = Maximum load
                  A = Indent area

Equation 2.   E = Elastic (Young's) modulus
                  S = Stiffness in elastic region of unloading
                  A = Indent area

$$ H = \frac{P_{max}}{A} $$

$$ E = \frac{S \cdot \pi^{1/2}}{2 \cdot A^{1/2}} $$

In the nanoindentation technique performed for the experiment, the hardness was measured at the end of the 30 second maximum load hold time, while the Young's modulus was

measured from the initial slope of the unloading curve for each indentation specimen. The nanohardness data accounts for not only elastic deformation, a property of the material, but any plastic (irreversible) deformation. On the other hand, Young's modulus is a measure of the stiffness of a material, measured in the elastic region of the unloading curve.

Nanoindentation was chosen as the indirect method of testing fluoride content for a few reasons. Firstly, Akimoto et al. (2001) and Meredith et al. (1996) demonstrated that accurate nanoindentation testing could be done on dentin specimens, even when affected by demineralization.[24,25] Indeed, the measured values for hardness and Young's modulus shown in Table 3, although a bit low, are still within the expected range for dentin.[24] Secondly, the original electron microprobe specimens could be preserved and used for the testing. Thus, the fluoride content of each specimen could be directly compared to the resistance to demineralization. Finally, in contrast to many other indirect methods of testing fluoride uptake, nanoindentation produces quantifiable results. The use of an atomic force microscope possibly could have produced more accurate data compared to the nanoindenter. In fact, Marshall et al. (2001) successfully used an atomic force microscope to measure the nanomechanical properties of carious dentin.[26] However, because atomic force microscopes are very sensitive instruments and are complicated to access and operate, the decision was made to use a nanoindenter instead. In retrospect, an entirely different method of indirectly measuring fluorine content may have been prudent. Techniques such as microradiography or polarized light microscopy of thin, demineralized sections may have produced more definitive results. However, these techniques also would have required thin sectioning of the specimens or the fabrication of new test specimens. Furthermore, the data gathered from techniques such as microradiography or polarized light microscopy generally only show zones of demineralization inhibition and would not be as potentially quantifiable as nanoindentation techniques.

Nanohardness and Young's moduli testing results demonstrated no significant trends or differences in the mechanical properties at the surface of the specimens. It is certainly possible that the specimens having increased levels of fluorine (as measured with the electron microprobe), may have been no more resistant to demineralization than specimens with minimal or no increase in fluorine content. Alternatively, the demineralization sequence may have been too modest, as the specimens were exposed to a 0.1 M buffered acetic acid (pH 4.5) immersion for only 1 minute. This minimal acid challenge did not affect the specimens to any degree that

was measurable with the chosen nanoindenter instrument and settings. It was expected that if the demineralization sequence had a meaningful effect on the specimen surface properties, there would be a difference seen between the 30 day undemineralized control group and the same group after the demineralization sequence (30 day demineralized control group). In fact, there was no significant measurable reduction in surface hardness or modulus after the demineralization sequence. Another possible explanation is that there were surface changes caused by the demineralization sequence, but the nanoindentation testing was not sensitive enough to detect such changes, which may have been primarily associated with the dentin tubules and peritubular dentin.

Despite the minimal hardness and modulus differences between specimen groups, some of the results of the nanoindentation are still difficult to account for. Interestingly, the hardness and Young's moduli data for one specimen group—the 30 day group—appears to be lower than the other groups. No clear explanation may account for the lowered mechanical properties measured with this group. It was originally believed that some variation in the data, especially for the 30 day (Fuji II LC-restored) specimens, could be a result of hydration or dehydration. It seems logical to assume that the 30 day containment of the specimens in 100% humidity may have resulted in more hydration of the specimens compared to the 1 day and 15 day specimens. However, with this assumption, the 30 day demineralized control specimens should have had similar hardness and moduli results. This was not seen in the data.

A study by Habelitz et al. (2002) reported on the effect of storage conditions of teeth on the nanomechanical properties.[27] In the study, it was found that storage of teeth in deionized water resulted in a large decrease in elastic modulus and hardness. After 1 day, an approximately 30% reduction was seen in dentin, and after one week, a 50% reduction was seen. The authors attributed the drop to a demineralization process during storage, as immersion in Hank's balanced salts solution did not significantly change the mechanical properties. The theory presented by the authors is that deionized water contains no calcium or phosphate ions and therefore will cause dissolution of these species in dentin at a conventional pH of around 6.5. In the methods used in this present study, each specimen was removed from a 100% humidity environment, sectioned and polished, and stored dry overnight until electron microprobe analysis could be performed. Subsequent to analysis, each specimen was repolished and stored dry for a period of 10-12 hours, at which time the specimens were subjected to the 1 minute

demineralization solution and then air dried for a few hours before mechanical testing was performed. It may have been prudent to store the specimens in an aqueous environment in the interval between the completion of electron microprobe analysis and nanoindentation. In fact, it has been reported in the literature that tooth specimens prepared for mechanical testing are often stored in an aqueous environment in order to maintain hydration.[27] Nevertheless, an average total dry (room air) storage time of approximately 36 hours elapsed between removal from the 100% humidity environment and nanomechanical testing. This time lapse was planned and kept consistent among the experimental groups.

For a few of the specimen groups, certain average values for hardness and modulus appear either too high or too low relative to the other values. Table 3 displays higher standard deviations at these specific distances. Despite careful testing it is possible that some indentations were made on or near a dentin tubule, which may have been just beneath the surface and undetectable using the optical light microscope. In instances where a dentin tubule is incorporated into the indentation process, the expected mechanical properties would be much lower. As the indent is made, the tubule absorbs a portion of the load, and gives less resistance. This may account for some of the dips in nanohardness and modulus values. A dilemma that was encountered during the demineralization sequence of the experiment was the enlarging of dentin tubules and the roughening of the specimen surfaces. Overly demineralized surfaces presented a problem in using nanoindentation to measure surface hardness and moduli. The literature reports that dentin tubules range from 1-3 μm in diameter and on average, there are 30,000 tubules per mm^2.[28] Per 10 μm^2, therefore, there would be an average of around 3 tubules. At 1-3 μm in diameter, this presents minimal problems when the dentin is unaltered. However, after demineralization, the tubules can become enlarged. During a demineralization process, the dentin tubules may be more susceptible to degradation as they often contain loose mineral deposits.[26] Figures 10 and 11 demonstrate the differences in tubule dimensions before and after acid attack. It should be pointed out that the observed tubule density in these micrographs does not appear to follow the aforementioned 3 tubules per 10 μm^2. This is likely due to the orientation of the specimen sections, which ran fairly parallel to the expected tubule orientation. Thus, the tubules seen in the Figures 10 and 11 run in an oblique direction to the specimen surface.

Since studies have reported the preferential diffusion of fluorine through the dentinal tubules within dentin,[29] it is reasonable to expect that the peritubular dentin may have favored fluoride uptake compared to intertubular dentin. These areas of peritubular dentin may account for a large proportion of the increases in dentin fluorine content seen in the electron microprobe analysis. Unfortunately, during the nanoindentation procedures, an attempt was made to avoid indenting over and around dentin tubules, which would skew the data because of their irregular surface. Although Marshall et al. (2001) demonstrated that the intertubular dentin provides a good representation of the mechanical properties of the dentin as a whole,[26] with preferential deposition of fluorine into peritubular dentin, there may be little or no measurable change in mechanical properties of demineralized specimens when primarily positioning indentations between dentin tubules, in the intertubular dentin.

CONCLUSIONS

The conclusions that can be made from the electron microprobe analysis of in vitro Fuji II LC-restored specimens are:

1) Fluorine concentrations at the tooth-restorative junction of Class V cavities restored with Fuji II LC increased from a period of 1 to 15 days in vitro. There was no increase in fluorine concentration noted between 15 and 30 days.

2) Depth of penetration of fluorine into dentin increased with time from 1 to 30 days from the tooth-restorative interface of the Class V restorations. Penetration of fluorine from the restorations reached over 30 μm into dentin after 30 days of in vitro maturation.

3) Significant differences ($p < 0.05$) in dentin fluorine concentration were found at locations up to 22 μm from the tooth-restorative interface for specimens matured for 30 days compared to specimens matured for only 1 day.

The conclusions that can be made about the demineralization and nanomechanical testing results are:

1) Increased dentin fluorine concentrations could not be correlated to an inhibition of demineralization using the chosen acid challenge of 0.1 M buffered acetic acid and Nanoindenter XP mechanical instrumentation.

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2) The chosen surface demineralization challenge followed by mechanical testing with a nanoindenter did not provide adequate surface conditions to accurately measure properties such as hardness and Young's moduli.

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