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Inhibition of decalcification around orthodontic appliances

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INHIBITION OF DECALCIFICATION AROUND ORTHODONTIC APPLIANCES

by

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THESIS

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ABSTRACT

Enamel demineralization around appliances during active orthodontic treatment remains a significant problem. The purpose of this *in vitro* study was to evaluate the optimal fluence (energy / surface area) of a 9.6 μm CO_2 laser that interacts with the labial surface of the teeth to inhibit demineralization without overheating the pulp. Orthodontic brackets were bonded to 44 extracted human teeth. On thirty-five teeth, brackets were bonded with Ormco system + 1 adhesive and Rely-a-Bond resin paste that do not have fluoride in their chemical component and on nine teeth brackets were bonded with Fuji glass ionomer adhesive and paste that has a high concentration of fluoride. Half of the exposed labial surfaces of the 35 teeth were treated with a 9.6 μm laser at fluences of 3, 4, or 5 J/cm^2 (experimental side) and the other half remained untreated (control side).The nine teeth in the glass ionomer group were treated with the laser at 4 J/cm^2 on the experimental side and the control side remained untreated. Teeth were cycled in *in vitro* demineralization and remineralization solutions the combination of which was previously shown to mimic the oral environment challenges and create similar caries-like lesions. All teeth were sectioned and examined for mineral loss by cross-sectional micorhardness testing. Approximately 50% inhibition of decalcification occurred with laser treatment alone when the experimental and the control sides in each group were compared except for the group that was treated with fluence of 5 J/cm^2. A reduction in decalcification of 83% was seen in the control region of glass ionomer group that contained fluoride when compared to the control region of the group (4 J/cm^2) that did not have fluoride in the bonding material. The group of teeth that was irradiated with a fluence of 5 J/cm^2 showed enamel surface defects such as ablation on the surface together with higher subsurface demineralization. The results of this study indicate that a low fluence of 3 J/cm^2 at a 9.6 μm is quite effective at inhibition of decalcification and maintaining the integrity of the enamel surface.

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One area of interest that has been investigated by dental researchers for the past decade is how to treat enamel surface with a laser to inhibit decalcification. Early investigations of the laser for treatment of dental hard tissue were discouraging because lasers were not available that operated at the frequencies required for strong absorption by dental hard tissue. High irradiation intensities were needed to change the enamel surface resulting in extensive structural cracking and thermal damage, e.g. charring, to the surrounding enamel and dentine after irradiation and eventual loss of pulpal vitality by overheating the pulp (Wigdor et al., 1995).

Several recent studies have demonstrated that the CO_2 laser can be used for prevention of decalcification and use of this laser within certain parameters can prevent injury to the pulp. CO_2 laser treatment of dental enamel can inhibit caries-like progression from 10-85% (Featherstone and Nelson, 1987). It is our hypothesis that treatment of enamel with specific laser parameters around orthodontic appliances can partially inhibit decalcification and protect the surface from formation of white spot lesions without excessively heating the pulp. This research will advance our understanding of which CO_2 laser irradiation fluences of 3, 4, or 5 J/cm^2 optimally inhibit decalcification without overheating the pulp.

A.2 Literature review

A.2.1 Proposed laser system for caries inhibition: CO_2 laser

Early results with the CO_2 laser on dental hard tissues were discouraging because those studies used continuous wave lasers operating at a wavelength of 10.6 μm, resulting in extensive peripheral thermal damage (Ferreira et al., 1989). However recent studies using pulsed CO_2 lasers have demonstrated that dental hard tissues can be efficiently thermally modified with minimal peripheral heat deposition in the tooth. Featherstone and

Nelson, (1987) showed that the 9.3 μm and 9.6 μm wavelengths were more efficient at heating the enamel surface than the 10.6 μm wavelength (Featherstone and Nelson, 1987).

The carbon dioxide laser produces radiation which falls in the infrared region and coincides closely with some of the absorption bands of carbonated hydroxyapatite (Nelson and Featherstone, 1982; Featherstone and Nelson, 1987). Dental enamel, dentine and cementum contain carbonated hydroxyapatite that has absorption bands in the area of infrared region due to phosphate, carbonate and hydroxyl groups in the crystal structure. The absorption coefficient of carbonated hydroxyapatite is higher than any other laser wavelengths throughout the visible, IR, and UV spectrum (Duplain et al., 1996) including the other CO_2 laser wavelengths of 9.3, 10.3, and 10.6 μm.

Kuroda and Fowler (1984) reported structural and phase changes in surface enamel when treated with the carbon dioxide laser which makes the enamel more resistant to acid dissolution (Kuroda and Fowler, 1984). All of these studies suggest that the carbon dioxide tuned to the highly absorbed 9.6 μm wavelength could be the system of choice for prevention of dental caries.

A.2.1.1 Basic laser physics

The first laser, or "maser" as it was initially called, was developed by Theodore H. Maiman of Hughes Aircraft Corporation in 1960. " Maser", like the more familiar term " laser" is an acronym for "Microwave Amplification by Stimulation Emission of Radiation" which describes the principle by which a laser operates.

The basic units, or quanta, of light are called photons. It is useful to think of quanta as particles. Quanta of electromagnetic energy are classified as cosmic rays, gamma rays, x-rays, light, microwaves, or radio waves. Light may be ultraviolet, visible or infrared. The physical property that determines this classification of electromagnetic energy is wavelength: the distance that a photon travels while the electric field completes one

oscillation. With current technology, laser light covers the range from about 0.1 to 11 μm. Short wavelength, e.g. ultraviolet radiation, is more energetic than long wavelength, e.g. infrared radiation.

A laser beam has a distinct spatial profile. The diameter of the beam , spot size, is used to calculate the proper incident energy of laser per unit area, defined as the incident fluence.

The process of stimulated emission occurs when an excited atom is stimulated to emit a photon before the process occurs spontaneously. When a photon of exactly the right wavelength enters the electromagnetic field of an excited atom, the incident photon triggers the decay of the excited electron to the lower energy state. This is accompanied by the release of the stored energy in the form of a second photon. The first photon is not absorbed but encounters other excited atoms. Stimulated emission can only occur when the incident photon has exactly the same energy as the released photon. Thus, the result of stimulated emission is two photons of identical wavelength traveling in the same direction oscillating in phase together. Each photon will trigger the release of two more photons and so on. In a small space at the speed of light, this photon chain reaction produces a brief, intense flash of light that has the same phase and same wavelength.

With the CO_2 laser the CO_2 molecules in the laser chamber are pumped to a higher energy vibration state and decay back to the ground state, releasing photons of a specific infrared wavelength.

A.2.1.2 Basic hard tissue optics

The biologic factors that influence laser-tissue interactions include optical properties of tissue elements which determine the nature of the tissue response through the processes of absorption, transmission, reflection and scattering of the beam.

At CO_2 wavelengths, scattering is negligible in enamel and dentine, and the energy deposition is determined by the absorption coefficient and tissue reflectance. Tissue reflectance of enamel has been shown to be 50 % for CO_2 laser radiation of 9.6 μm (Fried et al, 1997).

The effects of laser irradiation on tissue depend on the distribution of deposited energy inside the tissue. The absorbed energy in tissue is rapidly converted to heat in picoseconds, therefore, the temperature rise at each point in the exposed tissue is a result of the deposited energy distribution and any heat conductance away from this source. The temperature rise on the enamel surface is the fundamental effect determining the extent of changes in the morphology and chemical structure of enamel.

Ideally, laser irradiation effects should be confined to a thin surface region of enamel producing fusion, melting, and recrystalization of enamel crystals, with little transmission or scattering of heat to the dentine and pulp. Therefore, it seems logical to use the 9.6 μm wavelength of the CO_2 laser that has a high relative absorption coefficient to carbonated hydroxyapatite with minimal scattering.

The essential elements of laser light that determine its interaction with matter are the radiation wavelength, fluence, and the temporal characteristics of the laser beam, such as pulse rate and pulse duration. Manipulation of these variables will enable the operator to have precise control over the laser to achieve the desired tissue effect.

A.2.1.3. Wavelength

For wavelengths that are highly absorbed by enamel, the laser radiation will be efficiently absorbed close to the enamel surface and transformed into heat. Wavelengths that are weakly absorbed should be ineffective as the energy heats a much thicker layer of tissue to correspondingly lower temperatures. In such cases the light is mostly scattered or transmitted through the tissue, rather than absorbed. The visible and near IR, where very

low absorption is observed, are not useful for caries preventive treatments. Based on the above reasoning, the most likely laser for an efficient thermal interaction is the $C0_2$ laser at 9.3 or 9.6 μm wavelengths (Featherstone et al., 1995).

A.2.1.4 Pulse Duration

A pulsed laser is the laser of choice for the purpose of enamel treatment for caries inhibition. The pulsed laser allows for short bursts of energy that cause a rapid temperature rise in the tissue minimizing the total heat deposition in the tooth. Ideally, the laser pulse duration should be on the order of the thermal relaxation time, e.g. the amount of time it takes to dissipate approximately half of the energy deposited at the surface of the tissue. For a pulse duration longer than the thermal relaxation time, a large fraction of the absorbed laser energy is conducted away from the enamel surface into the interior of the tooth resulting in inefficient surface heating and possible pulpal damage. For pulse durations that are too short, the irradiation intensity may be too high causing ablation of tissue instead of the desired heating and fusion. Based on the thermal diffusivity of enamel the thermal relaxation time of a 10 μm enamel layer is approximately 60 μs. Therefore, a pulse duration in the range of 50-100 μs should be effective (Featherstone et al., 1995).

A.2.1.5 Pulse energy and fluence

The aim of the laser treatment is to raise the temperature of the enamel at and near the surface sufficiently to alter the mineral and make it resistant to acid dissolution without causing excessive ablation or undesirable peripheral damage. The precise temperature range that is desired has not yet been established, but from work with synthetic apatite and enamel, temperatures in the range of 400-1300°C are needed to beneficially modify the structure of the enamel to make it resistant to acid (Featherstone and Nelson, 1987).The

temperature rise in the tissue is commensurate with the laser irradiation intensity and pulse duration. Melting of enamel occurs in the range of about 900-1300°C and ablation occurs above 1600°C (Fried et al., 1995). From these results we would expect that fluences in the range of 2-6 J/cm^2 at the 9.6 μm wavelength would be most useful for reduction of enamel solubility, and that fluences of 10 J/cm^2 or greater could be harmful (Featherstone et al., 1995).

A.2.1.6 Repetition rate and number of pulse

The repetition rate needs to be sufficiently slow to allow for cooling in between pulses, but high enough to allow for clinically useful irradiation times. It was calculated that 10 Hz would fulfill both conditions. The number of pulses must be sufficient to provide the desired surface conditioning effect, to affect the immediate subsurface positively, but to minimize the total energy delivered inside the tooth to avoid overheating the pulp (Featherstone et al., 1995).

A.2.1.7 Proposed mechanism of inhibition

The enamel of intact human teeth irradiated with laser is known to have less subsurface demineralization than unirradiated enamel, on exposure to acid. Consequently, the potential use of laser irradiance to reduce caries is apparent. The laser induced physical and/or chemical changes that cause this reduced subsurface demineralization are not exactly known. A laser-irradiated tooth enamel surface will have a temperature gradient that decreases towards the dentine junction. Dependent on laser irradiation conditions, the temperature may range from >1200°C at the surface to near ambient at the dentine-pulp junction. Along this steep temperature gradient, different compositional, structural, and phase changes in the tooth enamel are to be expected. Changes in laser-irradiated material

from the highest temperature region have been characterized, but those occurring in sequential layers of decreasing temperatures have not.

High temperatures around 1200°C that exceed the melting point of enamel, may decrease the permeability of enamel. However, decomposition produces other phases that are more susceptible to acid dissolution. Lower temperature heat treatments (650°C) may increase the acid resistance by chemical changes, e.g. loss of organic components and carbonate. Kuroda and Fowler (1984) have shown that human enamel subjected to high energy density (10,000 J/cm^2), 10.6 μm wavelength of the carbon dioxide laser resulted in enamel-apatite melting. The melt was composed of minor phases of α-tricalcium phosphate, and tetracalcium phosphate. The apatite modifications, as compared with the original were; 1. reduced contents of water, protein, carbonate, and chloride; 2. essentially no change in apatite hydroxide content; 3. possible incorporation of oxide replacing some hydroxide ions; and 4. an uptake of traces of carbon dioxide and cyanate (Kuroda and Fowler, 1984). Nelson et al; (1987) conducted a study looking at the surface morphology of human enamel treated with pulse duration (100-200 nsec), infrared laser radiation (9-11 μm wavelength, 10-50 J/cm^2 cumulative fluences) using reflected and scanning electron microscopy (Nelson et al., 1987). The result showed thin (<5 μm) surface melts with varying degree of surface micro-roughening. Significant heat conductance under the surface melt was limited to a depth of approximately 10-20 μm. Tetracalcium diphosphate monoxide was identified as being a component of the surface melt together with an apatite phase that had a reduced carbonate content when compared to normal surface enamel.

A study by Featherstone et al. (1997) showed that irradiation of dental enamel by specific wavelength and fluences of carbon dioxide laser light alters the chemical composition of the crystals, decomposing the carbonate component, thereby markedly reducing the reactivities, and producing protection against the progression of subsurface caries-like lesions (Featherstone et al., 1997).

A comprehensive analysis of the relative importance of all these various chemical and morphological changes on the mechanism of acid resistance has yet to be determined. However, it is now known that laser treatment requires wavelength-specific laser irradiation for efficient conversion of laser energy into heat at the enamel surface and in the immediate subsurface, causing crystal transformation, loss of organic, and subsequent inhibition of subsurface lesion formation.

A.2.1.8 Peripheral thermal damage

Heat may accumulate in the tooth to dangerous levels during multiple pulse laser irradiation. Hence, it is necessary to measure the residual heat that is accumulated in the tooth per laser pulse during irradiation. The 1962, Zach and Cohen's study of the effect of heat on the pulp of Rhesus monkeys showed that a temperature rise of 5.5°C in the pulp caused irreversible pulpitis in 15% of the pulps (Zach and Cohen, 1962). This 5.5°C temperature rise is typically used as a temperature threshold that should not be exceeded. Higher temperatures may cause thermal damage to the pulp site. The accumulation of heat after use of the dental drill for cavity preparations and for the finishing of restorative materials can raise the pulpal temperature to dangerous levels if air/water cooling is not used (Miserendino et al., 1993). Similarly, the use of multiple pulse irradiation without air/water cooling may also result in the accumulation of heat to levels that could cause irreversible damage to the pulp. Subsurface thermocouple measurements, simulations of heat conduction, and histological examinations during laser irradiation show that the extent of pulpal heating is determined by the rate of deposition of the laser energy in the tooth, the distance of the laser spot to the pulp, and the rate of energy loss from the tooth (Boehm et al, 1975). Miserendino et al., (1993) observed that typically the temperature excursions in the pulpal wall occurred 10-20 seconds after irradiation of the surface of the tooth. Applying air/water cooling for five seconds after the five second continuous wave of 10.6

μm laser exposures of 10-50 J reduced the otherwise excessive temperature rise in the pulp chamber to a safe level. Miserendino et al., (1993) compared the temperature rise in the pulp chamber after depositing energy using a continuous wave $C0_2$ laser or a high speed rotary drill both with air/water cooling. They measured no significant difference in the temperature rise (Miserendino et al., 1993). Temperature excursions in the dental pulp chamber can be reduced by air/water cooling because of the slow rate of thermal diffusion from the surface of the tooth to the pulp chamber.

A.2.2 Demineralization of enamel around orthodontic appliances

Decalcification is defined as loss of tooth mineral. It occurs when the pH of the oral environment favors diffusion of calcium and phosphate ions out of the enamel. This dissolution follows the production of acid by bacterial plaque and results in an altered appearance of the tooth surface. The early lesion is an opaque white spot that, in active lesions, appears chalky. If mineral loss continues, frank cavitation may result.

An accumulation of bacterial plaque and a supply of fermentable sugars are the prerequisites for decalcification to occur. Unfortunately, fixed orthodontic appliances interfere with tooth cleaning, and favor plaque and food retention. A rise in the numbers of Streptococcus mutans and Lactobacilli has been reported following the placement of orthodontic appliances (Lundstrom and Krasse, 1987). These micro-organisms are associated with the initiation of dental caries and further development of the carious lesion, respectively, and their presence increases the risk of decalcification (Schwaninger and Vickers-Schwaninger, 1979).

Early studies in this field investigated the overall caries experience of orthodontic patients and found that although the total number of carious lesions did not differ significantly from untreated individuals, a greater proportion of buccal and lingual surface lesions were seen (Wisth and Nord, 1977). Subsequent workers have attempted to quantify

the extent of the risk posed by decalcification during orthodontic treatment. The prevalence reported among patients ranges from 2-96 per cent. This large variation is due to the variety of methods and populations of patients used to assess and score the presence of decalcification, whether idiopathic enamel lucencies were included or excluded, and the use or otherwise of a fluoride regime during treatment. It is difficult to distinguish between idiopathic white spots and decalcification, which artificially can increase the prevalence quoted in a cross-sectional study. Zachrisson (1977) employed a longitudinal design and recorded only new white spots developing. The prevalence of the new white spot lesions formed was reported to be 15 %, (Zachrisson, 1977).

A.2.2.1 The distribution of affected teeth

Gorlick et al., (1982) found maxillary incisors and mandibular first molars to have the highest prevalence of caries. Mizrahi (1983) found maxillary incisors and first molars to be most commonly afflicted. He also reported that the enamel opacities were found, particularly, on the cervical and middle thirds of the vestibular surface of affected teeth. Ogaard (1989) also found the first permanent molars in both arches to have the highest prevalence of caries. In contrast, Geiger et al., (1989), reported that caries lesions occurred most frequently on maxillary lateral incisors and canines, and on mandibular premolars.

A.2.2.2 Etiology of decalcification

The interdependence of bacteria, sugar, enamel, and time in the etiology of caries is well accepted. When fermentable carbohydrates are taken into the mouth, bacteria metabolize them producing weak organic acids such as acetic acid, lactic acid and propionic acid. These acids will diffuse readily through the plaque into the underlying enamel finding

susceptible sites on it. As the acids diffuse, mineral loss occurs on enamel and early carious lesion progresses (Featherston et al., 1990).

Additional factors can predispose enamel to decalcification. The presence of orthodontic attachments make tooth cleaning more difficult and predisposes it to a build-up of plaque on the surface (Ciancio et al., 1985). In addition, a layer of plaque on an enamel surface not only provides a source of acid production in the presence of a sugary substrate, but also acts as a physical barrier limiting the diffusion of acid away from the tooth surface, preventing remineralization from calcium and phosphate ions in the saliva. Given these factors, it its not surprising that decalcification is particularly associated with those areas where plaque accumulation occurs in the orthodontic patients (Saloum and Sondhi, 1987).

Frequent consumption of sugary foods or drinks has been shown to be most damaging to teeth. Following sugar intake, the pH of plaque drops below the critical level of 5.5 for about 20 minutes (Featherstone et al., 1990). Fixed appliances restrict the ability of the tongue and saliva to remove food particles from the mouth with the result that breakdown of more complex carbohydrates gives rise to a prolonged acid challenge to the tooth surface.

As the proportion of tooth surface covered by an orthodontic bracket increases, it becomes more difficult for the patient to effectively clean the remaining uncovered enamel. The presence of adhesive flash around a bonded orthodontic attachment can predispose enamel to plaque accumulation because of the rough surface on the enamel created by the adhesive flash (Gwinnett and Ceen, 1979).

A.2.2.3 Methods of reducing decalcification

Most operators insist on a patient attaining a certain level of oral cleanliness before embarking on orthodontic treatment. However, there will always be a proportion of patients whose ability to clean their teeth deteriorates as treatment progresses. Remotivation

and education of the patient in the importance of diet and tooth cleaning throughout treatment can help to reduce this problem.

Chlorhexidine is antibacterial and is effective due to its absorption onto the acquired pellicle, that prolongs its presence and effect in the mouth (Hogg, 1990).

Selection of small brackets and their use with a careful technique involving removal of any composite flash, minimal use of looped archwires, and periodic checking of the cement lute under the bands, will help to reduce plaque accumulation by the appliance and decalcification.

Fluoride has been shown to reduce caries by: 1. Acting as a catalyst favoring the formation of fluoroapatite; 2. Aiding remineralization during pH fluctuations; 3. Inhibiting glycolysis of plaque bacteria (Levine, 1991). Therefore, fluoride enhances remineralization of enamel caries and produces a mineral which is more resistant to subsequent demineralization. ten Cate and Featherstone in 1991 have shown the two principal steps that are involved in this mechanism. In the first step, partially demineralized crystals will grow new mineral on their surface when calcium, phosphate and fluoride are present in the surrounding solution. In the second step, the new mineral surface excludes carbonates and forms fluoroapatite which is a highly resistant mineral with a much lower solubility than the original hydroxyapatite (ten Cate and Featherstone, 1991).

Fluoride is used in almost all toothpastes available in the U.S.. For caries susceptible patients, different approaches have been employed to increase the availability of fluoride ions around orthodontic attachments. Daily mouth-rinsing with 0.5% sodium fluoride solution has been shown to be effective at reducing the prevalence of decalcification (Zachrisson, 1975). Professional fluoride gel applications during orthodontic treatment are of proven efficacy (O'Reilly and Featherstone, 1987). The drawback of these approaches is that those patients who would most benefit from topical fluoride are the least likely to comply with a regular rinsing regime.

Other means of protecting the enamel surface are applying varnishes containing fluoride around and beneath the orthodontic attachments, adding fluoride to cements for banding, bonding agents and to releasing modules. However, no clinical data has been published to show the continuous release of sufficient amount of fluoride over long-term of orthodontic treatment.

A.3 SPECIFIC AIMS

The overall objective of this study is to determine the specific laser irradiation fluence that most efficiently interacts with enamel and inhibits decalcification around the orthodontic appliances while minimizing the heat deposition in the teeth.

The hypothesis is that laser radiation tuned to the absorption maximum of carbonated hydroxyapatite near the 9.6 μm wavelength of the $C0_2$ laser, with a pulse duration that is on the order of the thermal relaxation for a 10 μm layer of heated tissue (100 μs), and fluences of 3, 4, or 5 J/cm^2 will prevent decalcification around orthodontic brackets. The specific aims are:

1. To carry out *in vitro* experiments to determine laser irradiation conditions that reduce the solubility of dental enamel. The following methods are employed to achieve this aim. (i) CO_2 laser conditions are selected on the basis of most promising results to date (ii) An *in vitro* pH cycling demineralization/remineralization model is used to create initial caries like lesions (iii) Microhardness test is chosen to measure the overall relative mineral loss from the enamel surface.
2. To establish the specific irradiation conditions that minimize thermal insult to peripheral healthy tissue. The following method is employed to achieve this aim for various irradiation fluences. (i) Microthermocouple temperature measurements will determine the heat accumulation in the tooth during multiple pulse laser irradiation.

B. EXPERIMENTAL DESIGN AND METHODS

B.1 Sample preparation and laser parameters

B.1.1 Sample selection and preparation for laser analysis

Extracted sound human permanent molars collected from an oral surgeon's office in San Francisco were used. Only noncarious and nonrestored teeth were used for the study. After extraction, teeth were stored in 0.02% thymol solution. Gamma radiation was used to sterilize them according to the protocols described in (White et al., 1994). Before commencing the experiment, teeth were brushed with a 1% detergent solution and residual soft tissue was cleaned from the cementoenamel junctions and root surfaces. Teeth were mounted on a tongue blade and roots were sectioned from the cementoenamel junction.

Crowns were inspected under the light microscope to choose enamel surfaces that were flat and were free of defects such as decalcification, fluorosis, cracks and chips. Subsequently, the selected crowns were mounted on a tongue blade and hemisected with fine diamond disks either buccal-lingually or mesial-distally depending on the surfaces selected that were without defect. Each hemisected surface was painted with acid-resistant varnish except for a flat surface which was left exposed on the enamel.

Pilot study #1: Ten hemisected crowns were used. The teeth were divided into two groups, five experimental (irradiated) and five control (non-irradiated). Five experimental teeth were irradiated with 4 J/cm^2 fluence (refer to section B.1.4). Orthodontic brackets were bonded to the middle of the exposed enamel. The area under the bracket was etched with 37% phosphoric acid for 15 seconds, rinsed for 20 seconds with water, dried for 10 seconds and a self cure adhesive was applied (Ormco system 1+, O). The adhesive was also applied to the bracket-base meshwork followed by bonding composite, (Rely-a-Bond, RB). This adhesive and bonding resin was chosen because no fluoride was present

in material. A bracket was placed on the prepared surface and adapted to the tooth. Excess composite around the bracket was removed. Hemisected crowns were cleaned with detergent and sonicated for 10 minutes. After pH cycling (refer to section B.1.3.3), these teeth were cut into the left and right halves through the brackets. Three areas were examined with microhardness measurements (refer to section B.1.6): occlusal, gingival enamel areas very close to the bracket, and under the bracket.

The following problems were identified in this pilot study #1. 1. The exact positioning of the bracket was not possible since, it was hard to delineate the laser-treated site. 2. It was difficult to restrict etchant and adhesive placement to under the bracket and thus, exposure of the surrounding enamel surface to etchant could have caused more mineral loss than expected. Adhesive contamination of the surrounding enamel could have falsely protected the enamel surface from showing any decalcification. 3. There was not enough room to examine the enamel occlusal and gingival regions of the bracket in some samples. 4. Irradiated and non-irradiated samples were from different teeth. Considering the variability that existed among teeth, it was decided to place the control and the laser treated surfaces on the same tooth.

Pilot study #2: Five hemisected crowns were used and each individual surface was divided into experimental (irradiated) and control (non-irradiated) halves. All teeth were irradiated with 4 J/cm^2 fluence (refer to section B.1.2). Orthodontic brackets were bonded to the middle of the enamel windows at the junction of irradiated and control surfaces. To restrict the effect of etchant and resin adhesive under the brackets, a piece of electrical tape was used to cover the enamel surface except for the area that the bracket was going to be placed. After isolation, the area under the bracket was etched with 37% phosphoric acid for 15 seconds, subsequently rinsed for 20 seconds with water, dried for 10 seconds, and a self cure adhesive (O) was subsequently applied. The adhesive was also applied to the bracket base meshwork followed by the bonding composite (RB). A bracket was placed on the prepared surface and was cemented to the tooth and excess composite

around the bracket was eliminated. After bonding occurred, the tape was removed. Hemisected crowns were sonicated for 10 minutes. After pH cycling (refer to section B.1.4), each section was further divided into upper and lower halves directly through the bracket. Each quarter consisted of a laser treated (irradiated) surface on one side of the bracket and its control (non-irradiated) on the opposite side of the bracket. Microhardness measurements were performed on cross sections of the irradiated and non-irradiated sides of the bracket and directly under the bracket.

The following modifications were implemented before the main study was initiated: Sections were irradiated after bracket placement to avoid interference of composite resin flash around the bracket. To prevent any interference from possible pellicle presence on the teeth, all samples were cleaned with 0.05% aluminum oxide suspension in deionized water.

Main study: forty-four hemisected crowns were used. These teeth were randomly divided into 4 groups. Orthodontic brackets were bonded to the middle of the exposed enamel. To restrict the effect of etchant and resin adhesive under the brackets, a piece of electrical tape was used to cover the enamel surface except for the area under the bracket. For the first three groups, the area under the bracket was etched with 37% phosphoric acid for 15 seconds, and the area was rinsed for 20 seconds with water, dried for 10 seconds and a self cure adhesive (O) was applied. The adhesive was also applied to the bracket base meshwork followed by a bonding composite (RB).

For the fourth group no etchant was used and a self cure glass ionomer, GC Fuji (GI), adhesive and cement were used for bonding the brackets to test the effect of fluoride release on decalcification. A bracket was placed on the prepared surface and cemented to the tooth surface. After bonding occurred, the tape was removed.

Each individual surface was divided into experimental (irradiated) on one side of the bracket and control (non-irradiated) on the other side of the bracket. The hemisectioned crowns were divided into four energy groups (for other laser parameters refer to section B.1.2):

1. The first group of 12 teeth were irradiated with 3 J/cm^2 fluence
2. The third group of 11 teeth were irradiated with 4 J/cm^2 fluence
3. The fourth group of 9 teeth were irradiated with 4 J/cm^2 fluence
4. The second group of 12 teeth were irradiated with 5 J/cm^2 fluence

Before pH cycling (refer to section B.1.4), to ensure that the enamel surfaces were clean and the pellicle was removed, the enamel surface of each hemisected crown was polished with 0.05% aluminum oxide slurry and sonicated in double deionized water for 10 minutes before pH cycling (refer to section B.1.4).

Groups:	***Pilot #1***	***Pilot #2***	***Main study #1***	***Main study #2***	***Main study #3***	***Main study #4***
Number of teeth:	10	5	12	11	9	12
Fluence:	4 J/cm2	4 J/cm2	3 J/cm2	4 J/cm2	4 J/cm2	5 J/cm2
Bonding Cement:	O, RB	O, RB	O, RB	O, RB	GI	O, RB

Table 1: Summary of the experimental design

B.1.2 Carbon Dioxide (CO_2) laser treatment

Pilot study #1: five teeth in the laser-treated group were irradiated with 4 J/cm^2 fluence over a 6x5 mm^2 area. The wavelength was tuned to 9.6 μm with a pulse duration of 100 μs. Using a 1 mm diameter beam, the area was scanned at 500 μm distances with a computer-operated micrometer-driven stage to provide uniform surface coverage.

Pilot study #2: Left-half segments of exposed enamel were irradiated with a CO_2 laser of 4 J/cm^2 fluence over a 6x5 mm^2 area. The wavelength was tuned to 9.6 μm with a

pulse duration of 100 μs. A 1 mm diameter beam was used and it scanned every 500 μm distance using a computer-operated micrometer-driven stage to provide uniform surface coverage.

Main study: Left half segments of the exposed enamel were irradiated next to the brackets with 9.6 μm laser radiation over a 5x5 mm^2area. A 1 mm diameter beam was used and it scanned every 500 μm distance using a computer-operated micrometer-driven stage to provide uniform surface coverage.

Fluences of 3, 4, 4, and 5 J/cm^2 per pulse were used for group #1, group #2, group #3, and group #4, respectively (refer to Table 1).

B.1.3 Preparation of demineralization and remineralization solution

The demineralization solution consisted of calcium phosphate (2 mmol/L) in an acetate (0.075 mol/L) buffer. The pH of this solution was adjusted to 4.5. Two batches of demineralization solution were prepared. The first demineralization solution was used to treat 12 teeth in group 5 J/cm^2 and 12 teeth in group 3 J/cm^2. Demineralization solution #2 was used to treat 11 teeth in group 4 J/cm^2 with Rely-a-bond cement and group 4 J/cm^2 with glass ionomer cement.

The remineralization solution consisted of calcium (1.5 mmol/L), phosphate (0.9 mmol/L), potassium chloride (150 mmol/L), and cacodylate (20 mmol/L) was prepared. The pH of this solution was adjusted to 7.0. This solution approximates the effect of calcium and phosphate from saliva and is similar to that utilized by (ten Cate and Duijsters, 1982).

B.1.3.1 Calcium analysis using atomic absorption (AA) spectrophotometer

Standard solutions of 0.5, 1.0, 2.0, 5.0 ppm were prepared by diluting the 1000 ppm calcium standard solution (Fischer calcium reference solution) with 1000 ppm KCl solution and were used to calibrate a Perkin Elmer 3110 Atomic Absorption (AA) Spectrophotometer. The presence of potassium insures that the calcium remains in its atomic state, as potassium will preferentially ionizes than calcium. Elemental calcium is necessary for the specific wavelength absorption of AA.

Samples of the demineralization and/or remineralization solutions were diluted ten times by KCl solution and analyzed using AA. Sample values measured by AA were multiplied by the sample dilution factor to calculate concentration of calcium [Ca] in demineralization and remineralization solutions in parts per million (ppm). The result shown in Table 2.

B.1.3.2 Phosphate analysis using UV spectrophotometer

Phosphate standard solutions of 0, 1, and 2 ppm and samples of demineralization and remineralization solutions were prepared with Reagent C that contained Ammonium Molybdate. Phosphorous and Ammonium Molybdate make a colored complex that absorbs light at 820 nm wavelength (Chen et al., 1956). A Milton Roy Genesys 5 UV Spectrophotometer was used at a wavelength of 820 nm. Phosphate standards and samples were placed in the spectrophotometer and values were read in absorption units. The standard values were averaged. The value of each sample that was read on the machine, was multiplied by the dilution factor and then divided by the standard mean value to obtain the phosphate concentration [P] and the results are shown in Table 2.

B.1.3.3 pH calibration

A Tim 900 pH stat was used to measure the pH. The machine was calibrated to pH values of 4 and 7 using pH standards from Fisher ChemAlert Guide. The pH of the demineralization and remineralization solutions were measured before and after the pH cycling (refer to section B.1.4).

Summary of calcium and phosphate concentrations and the result of pH analysis for remineralization solution (remin.) and demineralization solution (demin.) #1 and #2 are shown in Table 2.

	Expected pH	pH analysis before pH cycling	pH analysis after pH cycling	Expected [Ca]	[Ca] analysis	Expected [P]	[P] analysis
Remin. solution	7.00	7.00	7.00	60 ppm	58.8	27.9 ppm	28.0
Demin. solution #1	4.50	4.50	4 .50	80 ppm	81.9	60 ppm	59.5
Demin. solution #2	4.50	4.50	4.50	80 ppm	91.0	60 ppm	58.8

Table 2. Summary of the solution analysis

B.1.4 Creating *in vitro* decalcification

The laboratory pH-cycling enamel demineralization/ remineralization model was used (Featherstone et al., 1986). The *in vitro* pH model used was similar to that originally reported by ten Cate and Duijsters (1982). The duration of de-and remineralization periods,

the length of the overall challenge, and the strength of the demineralization solution were designed to give an end-result comparable to that found *in vivo* around orthodontic brackets (O'Reilly and Featherstone, 1987).

A strip of thin silk thread was attached to the bracket of each tooth and the tooth was suspended in the 40 ml of demineralization solution and subsequently in the 20 ml of remineralization solution. The teeth were placed in a demineralization solution for 6 hours and then washed with double deionized water two times and placed in remineralization solution for 17 hours. The teeth were stored at 37°C throughout the experiment. This cycling system was repeated daily on consecutive days for a total of 9 days of pH-cycling, interrupted during one weekend when the teeth were stored at 37°C in the remineralizing solution. The test scheme was designed to model a daily demineralization challenge of 6 hours, and a 17-hour remineralization. The extra hour allowed for manipulation of all the groups daily before and after demineralization. After the 9 days, teeth were stored in DDW until they were sectioned and prepared for the embedding. The pH of the solutions was measured before and after the 9 day of pH cycling.

B.1.5 Embedding and polishing

Each tooth sample was cut in half along the bracket slot, so that each half would consist of both control and irradiated enamel on either side of the bracket for the main study (the distribution of control and treated side and how they were sectioned were discussed previously for the pilot studies).

The interior of a PVC ring was greased carefully and taped at one end. The ring was placed on double sided tape on a metal tray. Samples were placed in a circular fashion inside the ring; brackets facing in and cut side down on the tape. The ring was marked as to identify the position of each tooth. Resin was prepared by mixing 20 grams of LX112, 14 grams of NMA, and 0.5 milliliters of DMP-30 (Ladd Research Industries). Resin was

poured slowly in the ring leaving slightly more than one cm from top of the ring. A dental explorer was used to go around the edges of each of the teeth to remove air bubbles prior to using the epoxy resin. The tray with each ring was placed in a 52°C oven overnight to cure the resin. One block was prepared for each pilot study and 7 blocks were prepared for the main study.

An Ecomet Polisher was used for surface polishing. The side of the block with the exposed cut faces of the teeth was polished with a 600 grade Silicon Carbide paper for 2 minutes. Double deionized water was sprayed on the polisher to prevent the surface from drying and to provide lubrication. The block was removed, washed with soap and brushed with a tooth brush in cold water. The block was sonicated for one minute, then washed again. The block was checked under the microscope for scratches. If most of the large scratches were removed the silicon carbide paper was removed and replaced with a 6 micron paper (Buehler) on the polisher. The surface of the polisher was sprayed by a 6 micron diamond suspension spray (Buehler). The block was polished for 4 minutes then washed and sonicated. The same procedures were repeated with 3 micron paper for three minutes and one micron paper for two minutes. The block was checked under the light microscope to make sure the scratches were removed. If scratches were still present, then the polishing procedure was repeated. For both the pilot studies and the main study, all of the samples were polished two times.

B.1.6 Microhardness measurements

After serially polishing the embedded teeth, the epoxy blocks were mounted on a microscope slides, using 5 minute epoxy gel. A small level was used to make sure the samples were flat. Samples were stained with a Rhodamine 6G dye (1%).

Each lesion was assessed by a microhardness examination using the Leitz Microhardness Tester. Magnification was set to low power. A 15 g indenter weight was

used, and the demineralized region (lesion) was located. Both sides of the microscope slide were taped down on the tester platform. A region, both free of cracks and air bubbles and with a straight enamel edge, was chosen for indentations. The accuracy of the diamond position was checked in the dentine. The outer edge of the specimen, i.e, the enamel-epoxy junction was identified. The first indent started at 15 μm from the outer surface, then four more indents moved horizontally 5 micron and vertically 50 micron, 3 more indents moving horizontally 5 micron and vertically 50 micron but in a direction opposite to the previous indents, followed by 10 indents moving 25 micron horizontally and 0 micron vertically. This provided a "stagger pattern" with indents at depths of 15 , 20, 25, 30, 45, 50, 75, 125, 150, 175, 200, 225, 250, 275 and 300 μm from the outer surface.

An image analyzer with Olympus lens, 500X magnification, was used to measure the length of the indents (Bioquant software). Indents were measured at the highest magnification (500X). It was important to measure the distances at which indents were made first to make sure the values were at correct distances on the sample.

The indentation lengths were converted via the Knoop hardness number, KHN, according to the following formula where K is the applied force in grams and L is observed indentation length in μm (Featherstone et al., 1983b):

$$KHN\ (Kg/mm^2) = 13230\ K / L^2$$

The Knoop hardness is converted to volume percent mineral according to the following formula (Featherstone et al., 1983):

$$Vol\ \%\ mineral = 4.3 \times (KHN)^{0.5} + 11.3$$

The conditions chosen were designed to produce control lesions approximately 100 μm deep with a mineral loss of up to 40%.

The overall relative mineral loss, ΔZ, for each lesion was obtained by subtracting the area under the hardness profile curve from the total 85 volume % mineral area for sound enamel. The area under the hardness profile curve as a function of distance from the enamel surface was calculated using Simpson's integration rule (White and Featherstone, 1987).

B.1.7 Statistical treatment of the data

Profiles were compared statistically between treatment groups and controls to assess their ability to inhibit caries progression using simple factorial ANOVA. The reduction in ΔZ value of the experimental group (irradiated) compared to the control group (non-irradiated) gives a measure of the efficacy of the laser pre-treatment. The means of the ΔZ values for each group were ranked and compared using the Fisher test ($p<0.05$) to determine the most successful set of conditions. Percent reduction in ΔZ mean value was calculated for each group as compared to the control group. Statview 4.5 was used for calculations.

Power analysis was used to determine the number of teeth in each group (Glantz, 1992). Results from the pilot study #2 were used to calculate the size of the treatment effect we wish to detect (∂), and the standard deviation (δ). Power calculations showed that with a sample size of 10 in each test group and risk of false positive, $\alpha = 0.05$, the power of the test would be 0.55. Three treatment groups in the main study contained more than 10 teeth to ensure that ten teeth would remain for the final analysis. In these groups the ability to detect a 40% change in the enamel surface is about 65%. Group GI started with 9 teeth and one tooth was lost. This group sample size of 8, yielded a power of 0.5 for a *t* test to detect a 40% change in the enamel surface.

B.1.7.1 Deficiencies and sources of error

In obtaining the sample, teeth were collected only from a single oral surgery's office. The limit in sampling means interpretation of the data to a general population will thus be conservative.

Enamel surfaces were reviewed under a light microscope to eliminate any surface defect. Defective enamel would most likely show more demineralization than normal enamel under the same conditions.

Technical errors such as slight contamination with adhesive or etchant could have occurred even though tape was used to cover the enamel surface during bracket placement. Contamination with adhesive could result in under-decalcification, while etchant could lead to over-decalcification.

Measurement error of indent lengths were within +/- 2% of volume % mineral measurements. This was calculated by measuring one indent ten times, finding the volume % mineral values and calculating the standard deviation.

ΔZ numbers were averaged over the number of teeth in a group and then compared among the four groups using single factorial ANOVA. There was significant variability among the teeth in terms of the relative mineral loss under the same conditions. Therefore, averaging ΔZs skewed the mean towards the larger numbers.

B.2 Microthermocouple measurement

The extent of pulp heating is primarily determined by the total heat energy deposition in the tooth, the distance from the laser spot to the pulp chamber; and the rate of energy loss from the tooth to the surrounding tissue. Low mass Teflon insulated microthermocouples (0.005 " Omega Type K), with a response time less than 100 ms were used to measure the subsurface temperature rise of 3 human teeth.

Each thermocouple was placed under the mesio-buccal pulp horn at specific depths from the surface. The teeth were imaged with X-Rays to determine the position of the thermocouple. The time resolved signal from the thermocouple was acquired by an oscilloscope and a PC based data acquisition system after being amplified by an Omega ONMI-I thermocouple amplifier.

The enamel surface at the top of the bracket in the middle of the buccal groove was irradiated with a CO_2 laser (10 times for one spot). The wavelength was tuned to 9.3 μm with a pulse duration of 100 μs, repetition rate of 10 Hz, and pulses of 25 per spot. The measurements were repeated 4 times per tooth. After each laser treatment and temperature measurement, water was used to reduce the temperature of the teeth. Laser irradiation was performed at 9.3 μm, because it was shown that both the 9.3 and the 9.6 μm have significantly high absorbtion coefficient for enamel (Featherstone et al., 1995).

Thermocouple output voltages are highly nonlinear. The voltage change per degree temperature change can vary by a factor of three or more over the operating temperature range of some thermocouples. For this reason the temperature from thermocouple voltages must be approximated by a polynomial equation. The polynomial equation is in the following form:

$$T = a_0 + a_1x + a_2x^2 + ... + a_nx^n$$

Where x is the thermocouple voltage in volts, T is the temperature difference between the measuring end and the cold junction in degrees Celsius, and a_0 through a_n are coefficients specific to each thermocouple type.

Subsequent to laser treatment and temperature measurement, teeth were cut horizontally along the plane of cementoenamel junction and vertically to expose the tip of thermocouple. The distance of thermocouple was measured to the irradiated area on the enamel surface with a caliper.

C. RESULTS

C.1 Effects of laser treatment

For each individual tooth, cross-sectional microhardness profiles were measured for three regions of under the bracket (UB) area, non-laser treated (NI) control area, and laser treated (I) area. Four energy groups of 3, 4, 4-GI, and 5 J/cm^2 were used to irradiate the laser treated region. The microhardness indent's lengths were converted to volume % mineral, and ΔZ (relative mineral loss) values were calculated (refer to section B.1.6).

Profiles were compared statistically between treatment energy groups and controls to assess their ability to inhibit caries progression. The reduction in ΔZ value of the irradiated (experiment) side of the tooth compared to the non-irradiated (control) side gives a measure of the efficacy of the laser. The reduction in ΔZ values were compared first among the 3 treatment regions for one fluence. Figure 1 shows a significant reduction in energy groups 3 and 4 J/cm^{2}and small reduction in groups 5 and 4-GI J/cm^2.

The percent reduction in ΔZ mean value was calculated for each energy group by comparing the laser treated region to the control region. The calculations show 42.6% reduction for the mean ΔZ value in pilot study #2. Energy group 3 J/cm^2 had 56.2 % reduction in mean ΔZ value. Energy group 4 J/cm^2 had 51.6 % reduction in mean ΔZ value. Energy group 4-GI J/cm^2 had 13 % reduction in mean ΔZ value and energy group 5 J/cm^2 had 22 % reduction. The means of the ΔZ values for energy groups were ranked and compared using the Fisher test ($p<0.05$) to determine the most successful set of conditions. This result is shown in the appendix. The result of this analysis shows that the energy group 4-GI was very successful with very little decalcification occurring overall. Energy groups 3 and 4 J/cm^2 where Ormco system 1+ and Rely-a-Bond used as a cementing medium also showed remarkable inhibition in decalcification compare to the control.

Interaction Bar Plot for Energy Groups
Comparing relative mineral loss (Error Bars:
95% Confidence Interval)

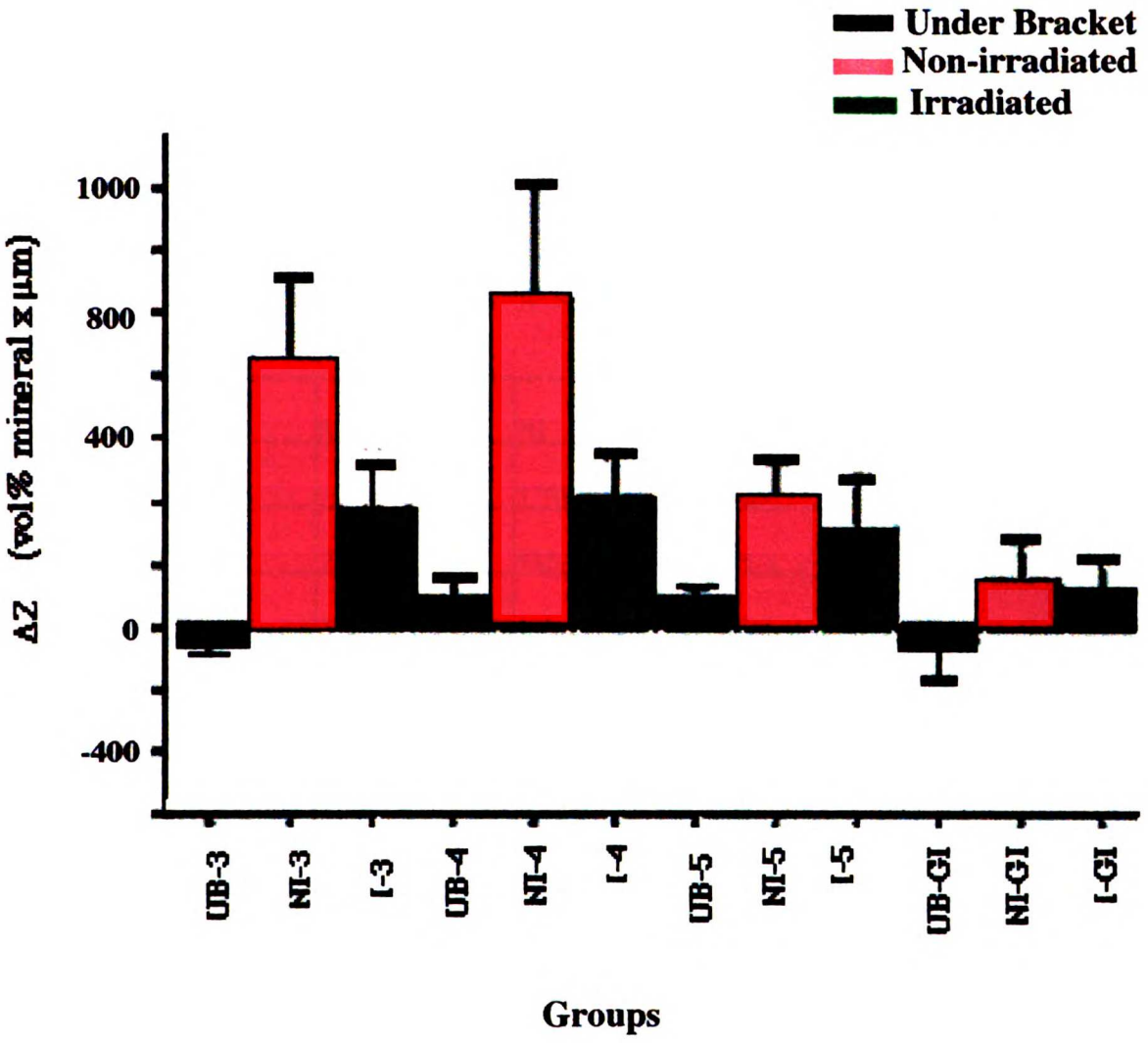


Fig. 1 Compares mean relative mineral loss of different regions under bracket (UB) black, non-irradiated (NI) red, and irradiated (I) green. Energy group 3 and 4 J/cm^2 show considerable inhibition of decalcification after laser treatment. Energy group GI, 4 J/cm^2 had very little decalcification in NI and I regions. Energy group 5 J/cm^2 had moderate amount of decalcification with little inhibition.

C.1.1 Pilot #2: Fluence of 4 J/cm^2

No significant results were found with pilot study #1.

Fig. 2 shows an example of indents in the non-irradiated control enamel under low magnification (50X). The same region at higher magnification (200X) is shown in Fig. 3. An example of indents in the laser treated region under low magnification (50X) is shown in Fig. 4 and at higher magnification (200X) in Fig. 5.

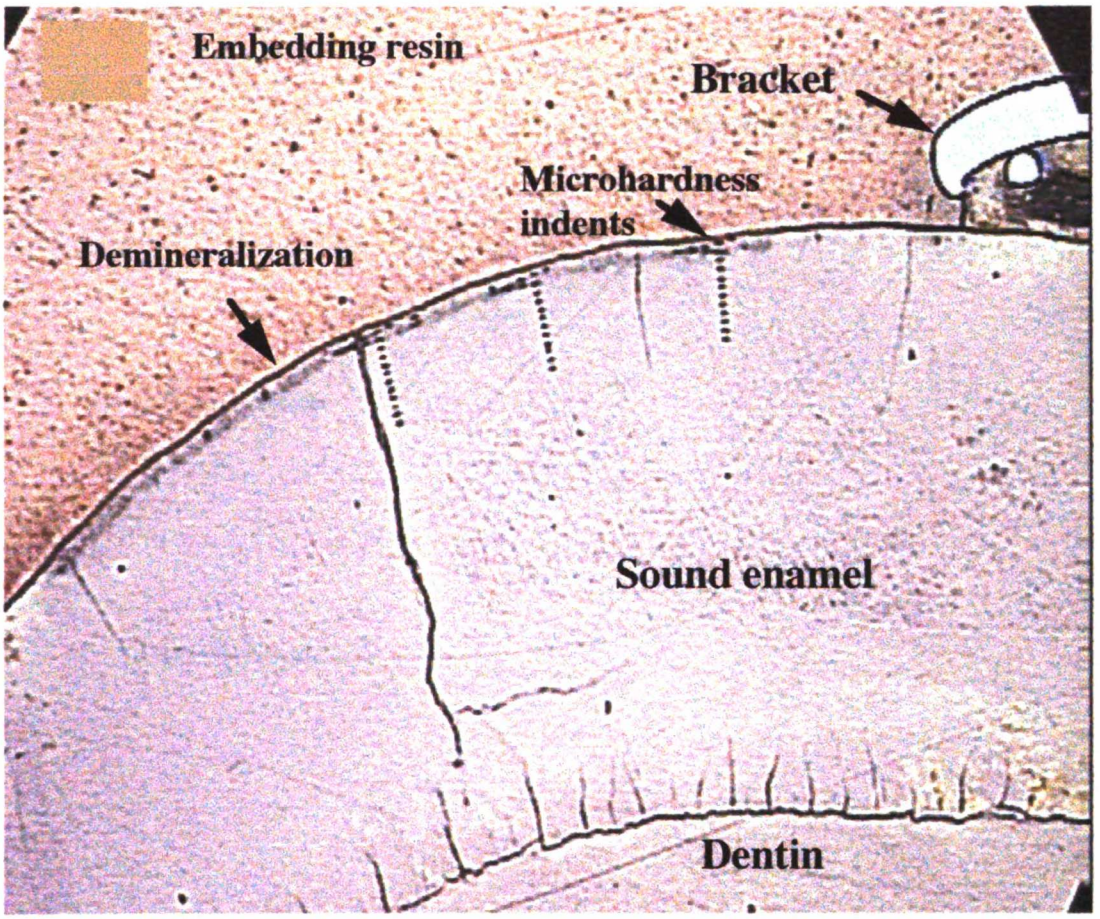
Descriptive statistics of ΔZs were performed and shown in Table 3. A negative value is an indication of more mineralization than 85% or falls within the measurement error. Reduction for mean ΔZ value from non-irradiated to irradiated regions is 42.6%.

	UB	NI	I
Mean ΔZ	-48.6	1701.7	975.9
Std. Dev.	76.5	825.4	284.1
Std.Error	44.2	369.3	127.1
Count	3	5	5
Minimum	-106.8	889.0	519.6
Maximum	38.0	2855.9	1234.4
#Missing	2	0	0

Table 3: Descriptive statistic for group pilot #2: 4 J/cm^2. Values are mean ΔZ (vol % X μm)

Average volume % mineral (ΔZ) was plotted against the distance from the enamel surface among the three regions of under bracket (UB), non-laser treated (NI), and laser treated (I). The result is displayed in Fig. 6 to show the change in area of relative mineral loss (ΔZ). Reduction of mineral was seen over the total area tested for the laser treated surface.

Pilot Study #2 (fluence of 4 J/cm^2): Non-irradiated control enamel after pH-cycling



- **Fig. 2** Non-irradiated enamel. Decalcification is the dark band just below the enamel surface. Three rows of 18 indents were placed to measure enamel hardness (50x)

Pilot Study #2 (fluence of 4 J/cm^2): Non-irradiated control enamel after pH cycling

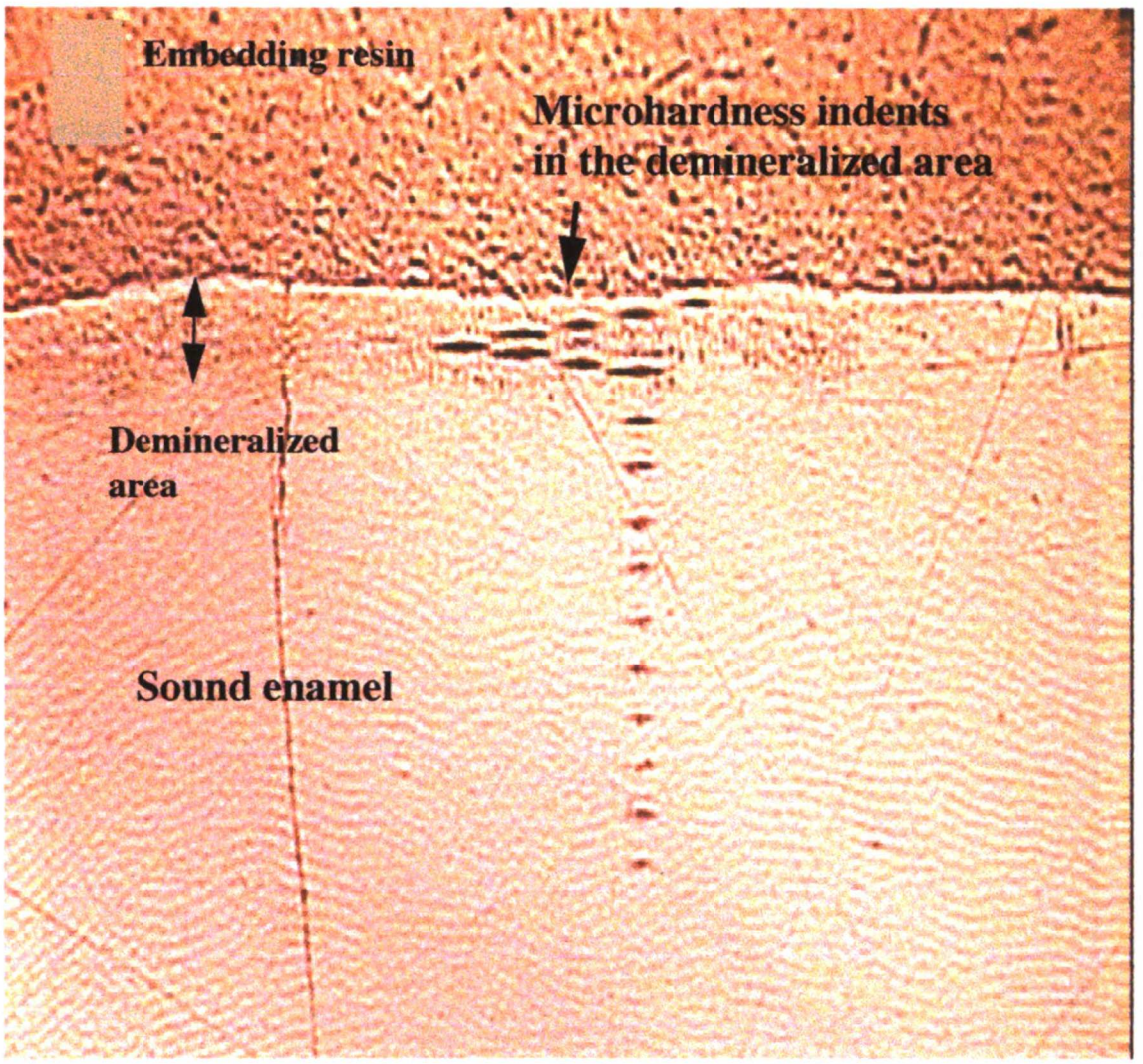


Fig. 3 Cross-section of non-irradiated enamel showing the indents (200x)

Pilot Study #2 (fluence of 4 J/cm^2): Irradiated enamel after pH-cycling

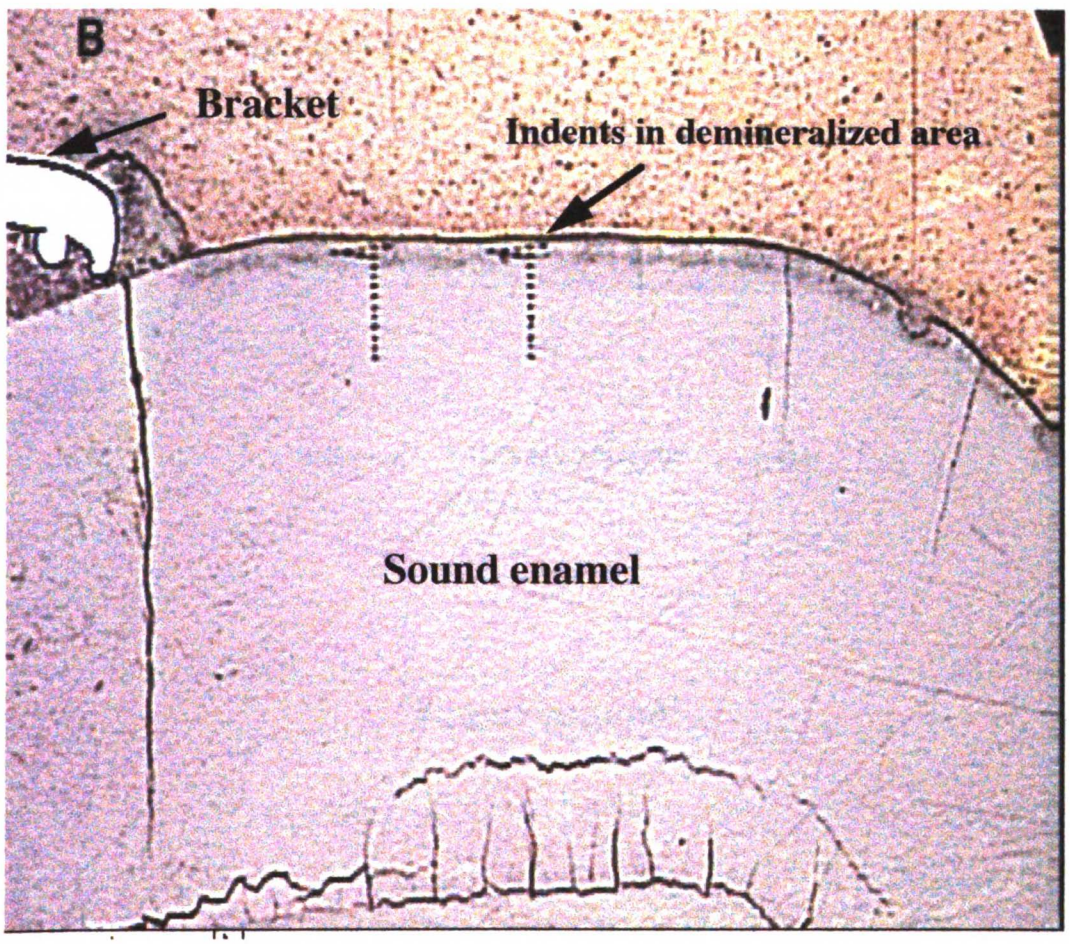


Fig. 4 Irradiated enamel with two rows of indents (50x)

Pilot Study #2 (fluence of 4 J/cm^2): Irradiated enamel after pH cycling

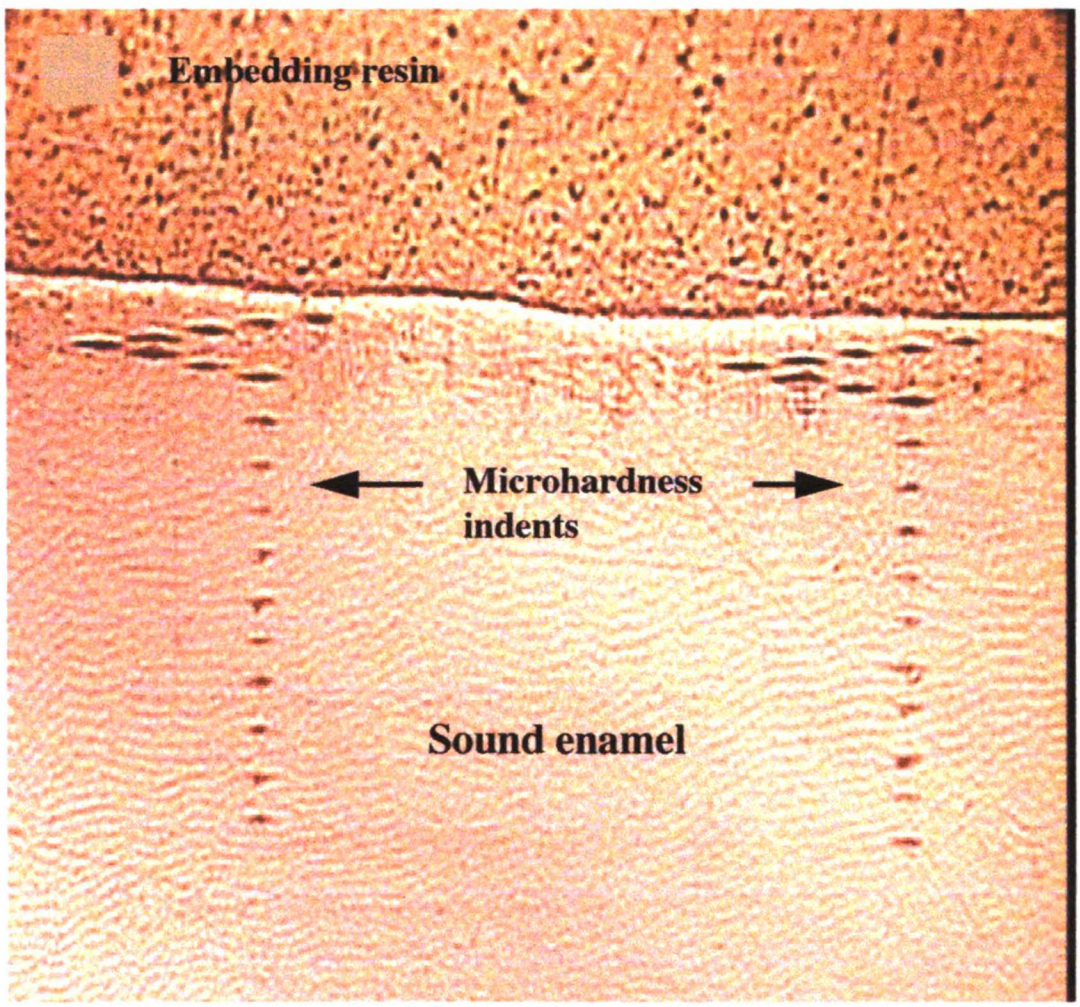
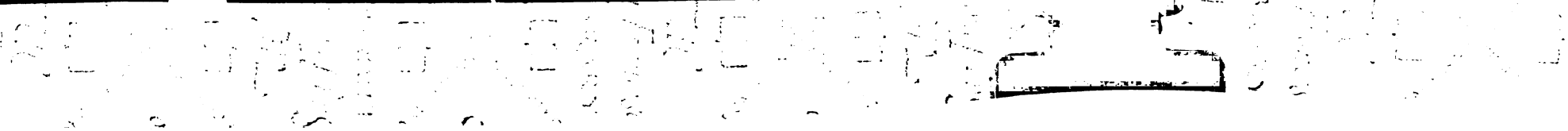


Fig. 5 Irradiated enamel showing little surface change and considerable inhibition of demineralization (200x)



Hardness Profile: Pilot Study #2 with fluence of 4 J/cm^2

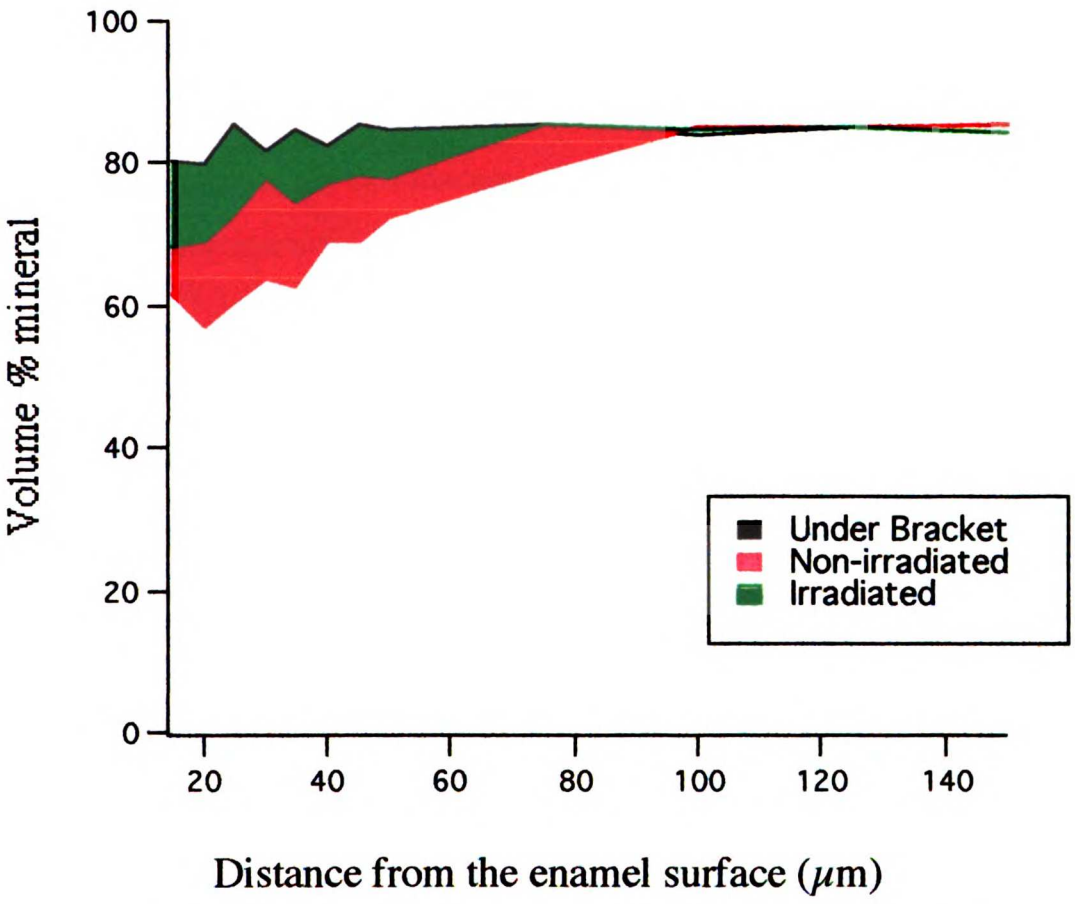


Fig. 6 Relative mineral loss of the irradiated region is less than the non-irradiated region. Lesion extends to the depth of 100 μm for the control region and to 80 μm for the laser treated region..

C.1.2 Main study #1: Fluence of 3 J/cm^2

Fig. 7 shows an example of indents in the non-irradiated control enamel under low magnification (50X). The same region at higher magnification (200X) is shown in Fig. 8. An example of indents in the laser treated region under low magnification (50X) is shown in Fig. 9 and at higher magnification (200X) in Fig. 10.

Descriptive statistics of ΔZs were performed and shown in Table 4. Reduction for mean ΔZ values from non-irradiated to irradiated regions was 56.2%. Statistically significant differences exist comparing the three regions to one another ($p < 0.05$).

	UB	NI	I
Mean ΔZ	-49.23	833.25	365.22
Std. Dev.	123.22	469.52	255.35
Std.Error	35.57	135.54	73.71
Count	12	12	12
Minimum	-245.30	48.10	81.60
Maximum	168.00	1955.70	927.10
#Missing	0	0	0

Table 4: Descriptive statistics for main study #1: Fluence of 3 J/cm^2. Values are ΔZ (vol% x μm)

Average volume % mineral was plotted against the distance from enamel surface among the three regions of under bracket (UB), non-laser treated (NI), and laser treated (I). The result is displayed in Fig. 11 to show the change in area of relative mineral loss (ΔZ). Reduction of mineral was seen over the total area tested for the laser-treated surface.

Main Study #1 (fluence of 3 J/cm^2): Non-irradiated control enamel after pH-cycling

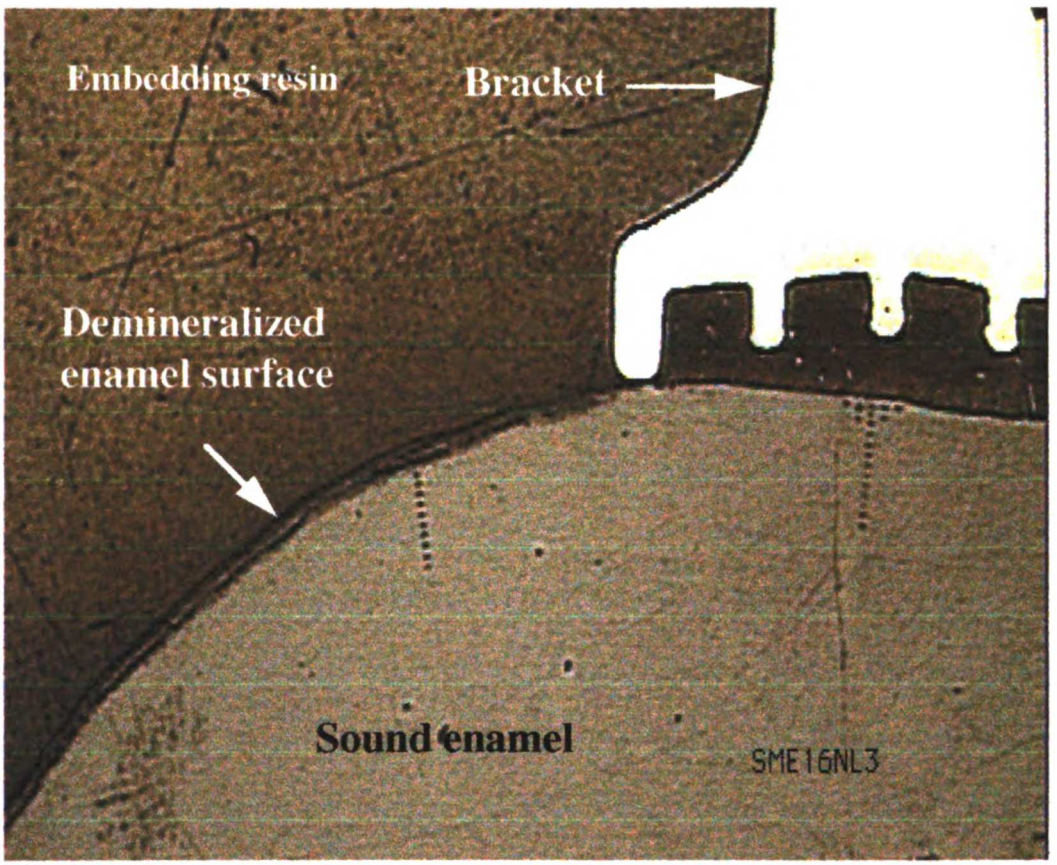


Fig. 7 Non-irradiated enamel showing dark zone of demineralization just below the enamel surface. Bracket is partially seen. (50x)

Main Study #1 (fluence of 4 J/cm^2): Non-irradiated control enamel after pH-cycling

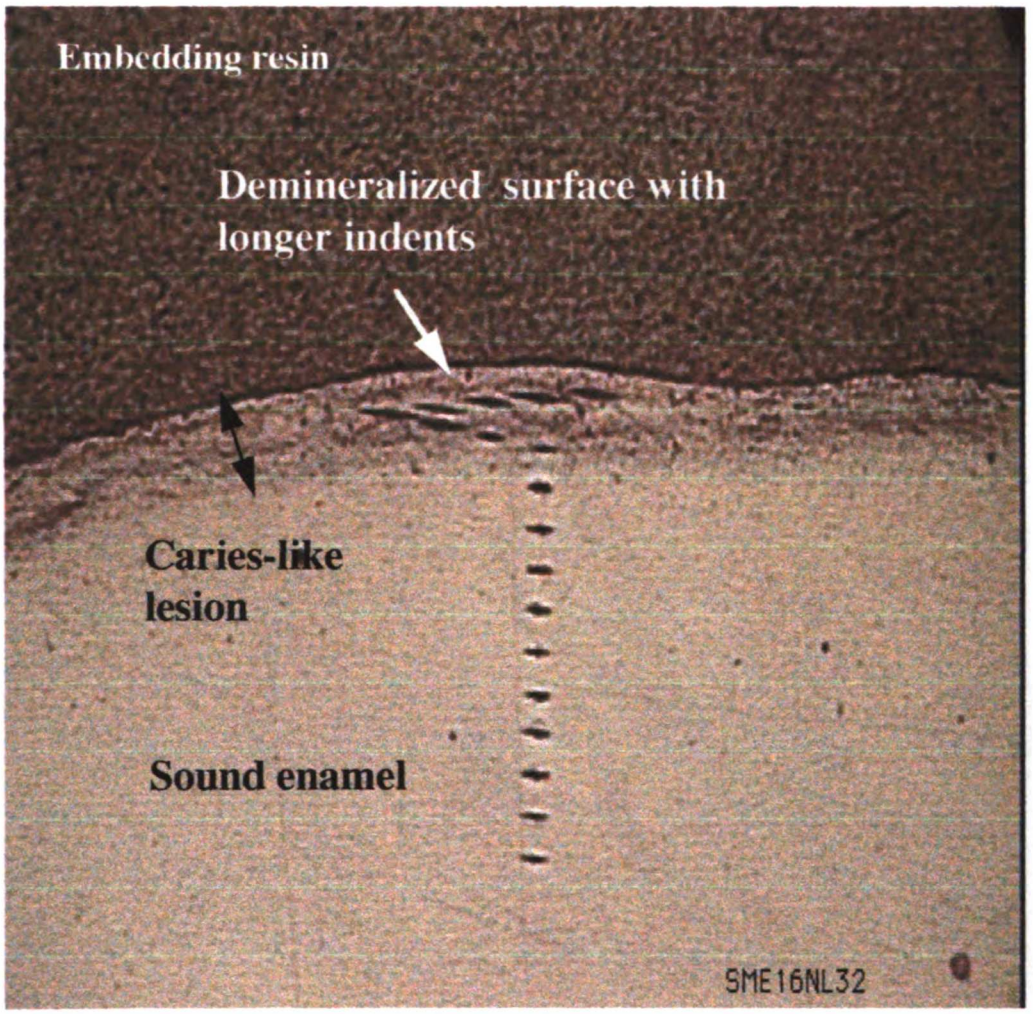
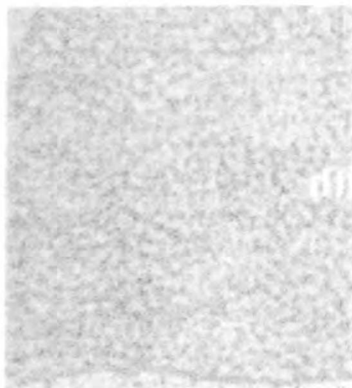


Fig. 8 Non-irradiated enamel showing heavy demineralization with longer indents just below the enamel surface (200x)



Main Study #1 (fluence of 3 J/cm^2): Irradiated enamel after pH-cycling

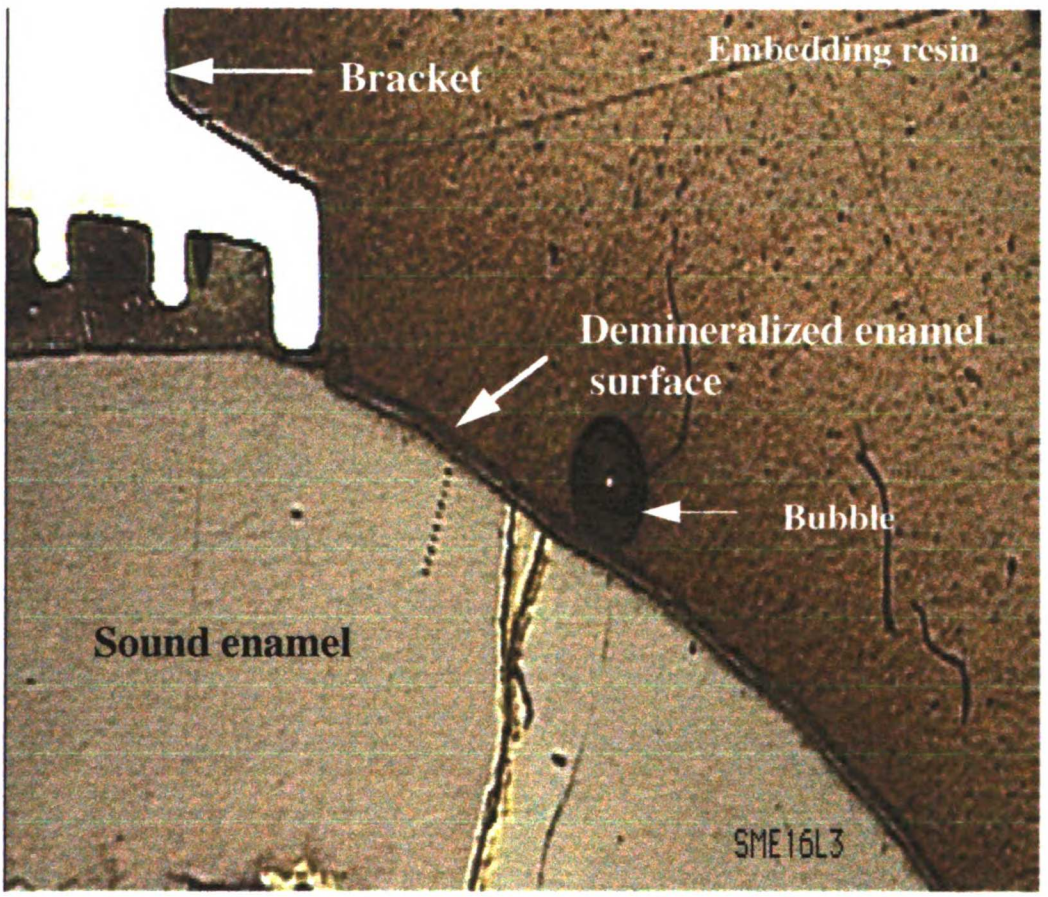
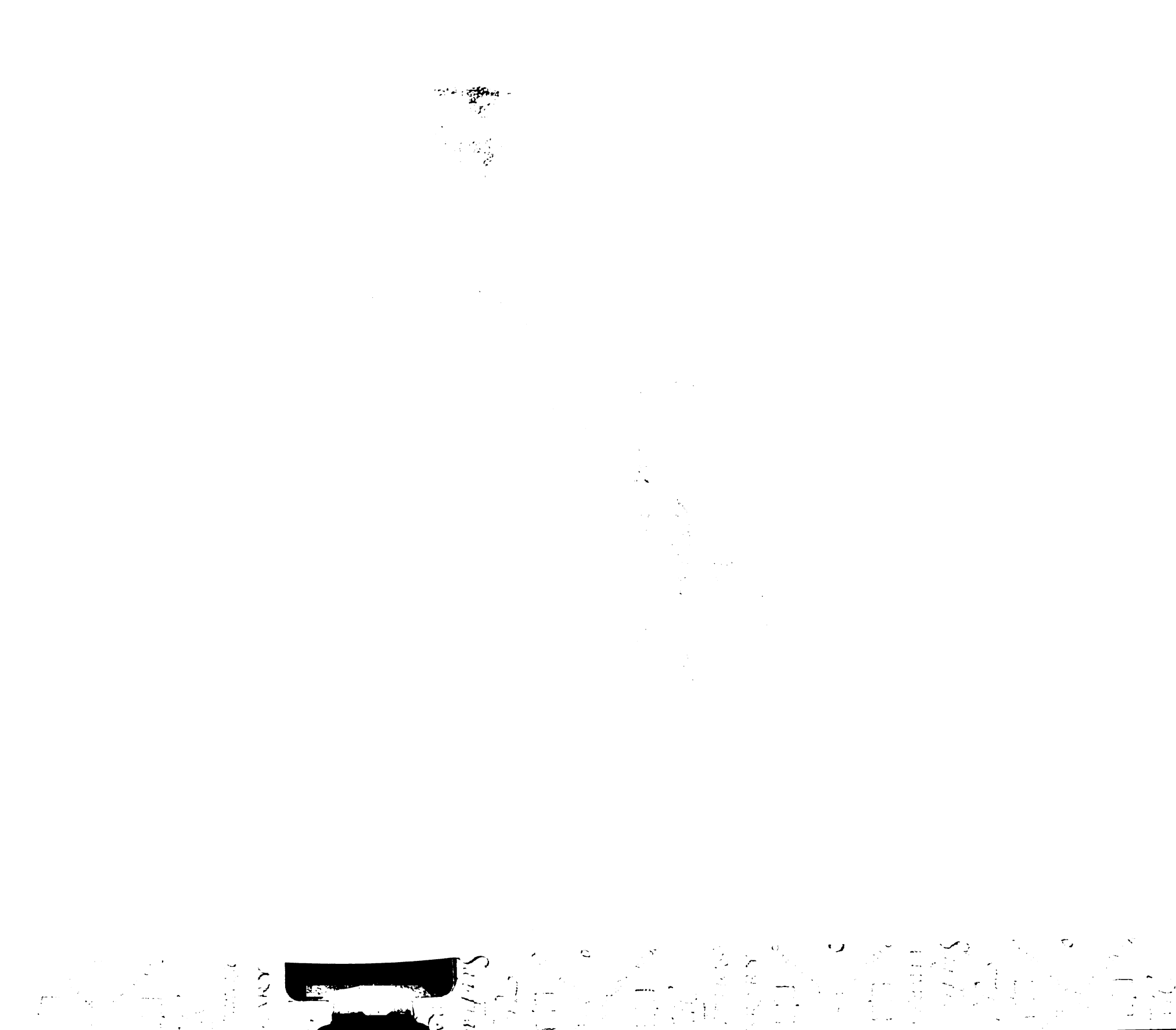


Fig. 9 Irradiated enamel showing narrow zone of demineralization just below the enamel surface (50x)



Main Study #1 (fluence of 3 J/cm^2): Irradiated enamel after pH-cycling

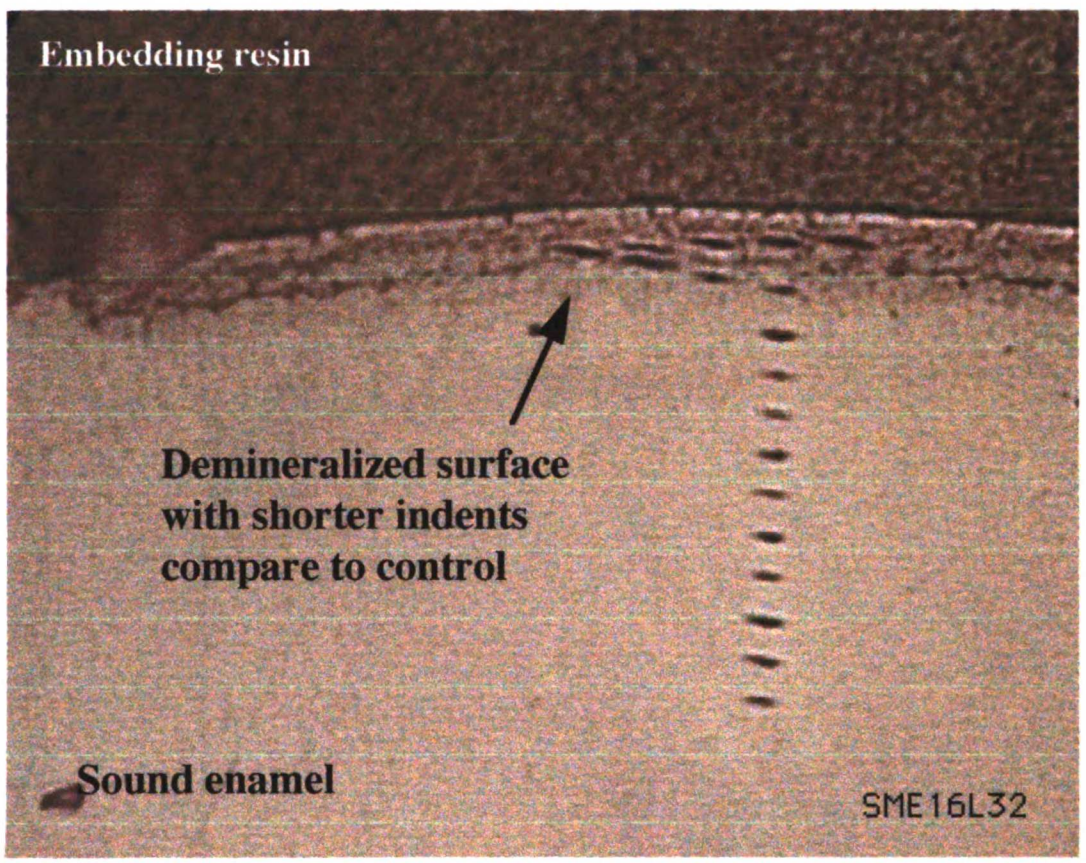


Fig. 10 Irradiated enamel showing smaller zone of demineralization compare to the control lesion (200x)

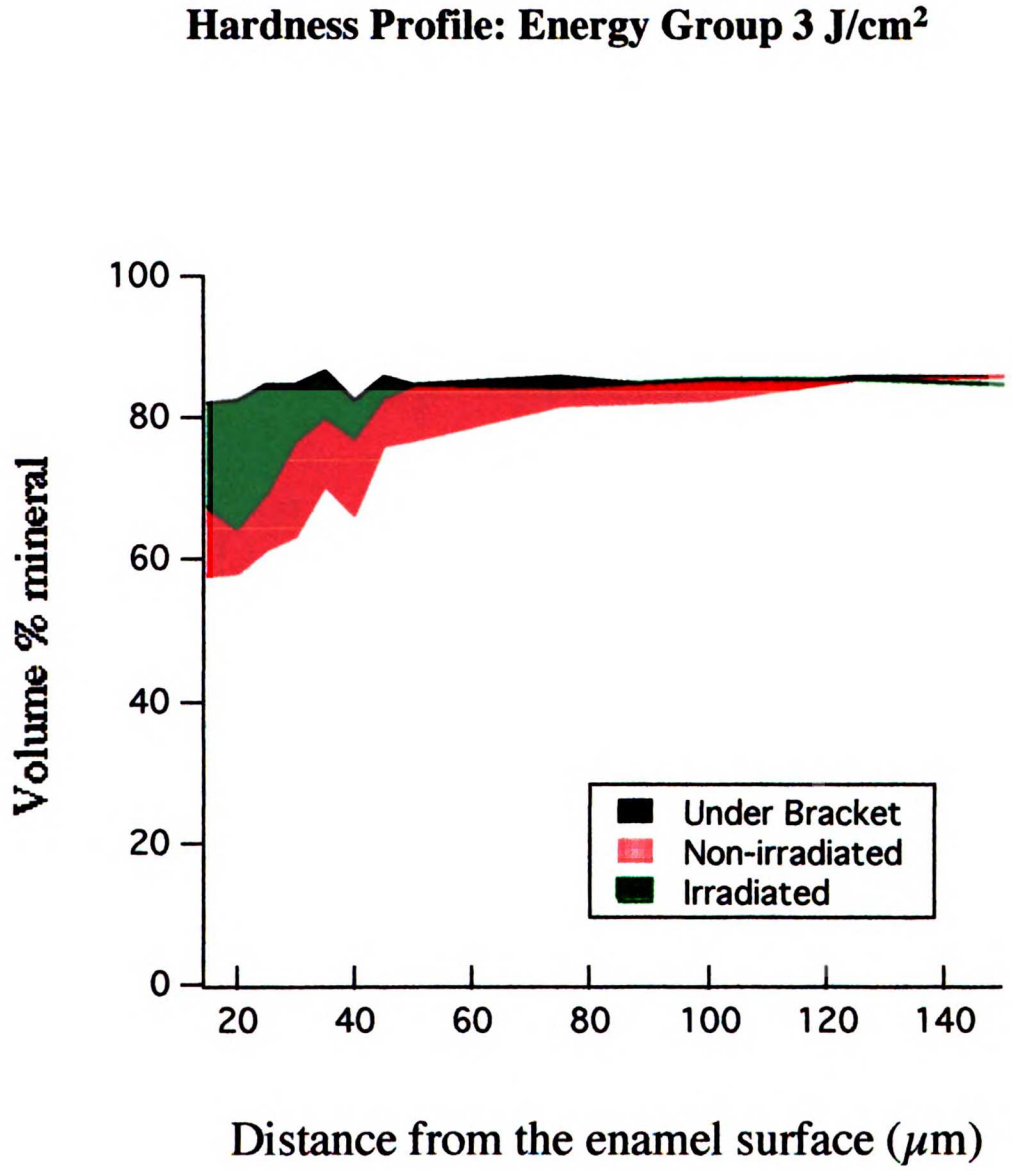


Fig. 11 Relative mineral loss of irradiated region is less than non-irradiated region over the whole tested area. Lesion extends to the depth of 120 μm for the control region and 90 μm for the laser treated region.

C.1.3 Main study #2: Fluence of 4 J/cm^2

Fig. 12 shows an example of indents in the non-irradiated control enamel under low magnification (50X). The same region at higher magnification (200X) is shown in Fig. 13. An example of indents in the laser treated region under low magnification (50X) is shown in Fig. 14 and its higher magnification (200X) in Fig. 15.

Descriptive statistics of ΔZs were performed and shown in Table 5. Reduction in mean ΔZ value from non-irradiated to irradiated regions was 51.6%. Statistical significant differences present among the three regions ($p < 0.05$).

	UB	NI	I
Mean ΔZ	78.07	1040.16	403.63
Std. Dev.	159.33	567.88	205.14
Std.Error	48.04	171.22	61.85
Count	11	11	11
Minimum	-104.90	142.60	131.00
Maximum	413.00	1946.00	897.20
#Missing	1	1	1

Table 5: Descriptive statistics for main study #2: Fluence of 4 J/cm^2. Values are ΔZ (vol% x μm)

Average volume % mineral was plotted against the distance from enamel surface among the three regions of under bracket (UB), non-laser treated (NI), and laser treated (I). The result is displayed in Fig. 16 to show the change in area of relative mineral loss (ΔZ). Reduction of mineral was seen over the total area tested for the laser treated surface.

Main Study #2 (fluence of 4 J/cm^2): Non-irradiated control enamel after pH-cycling

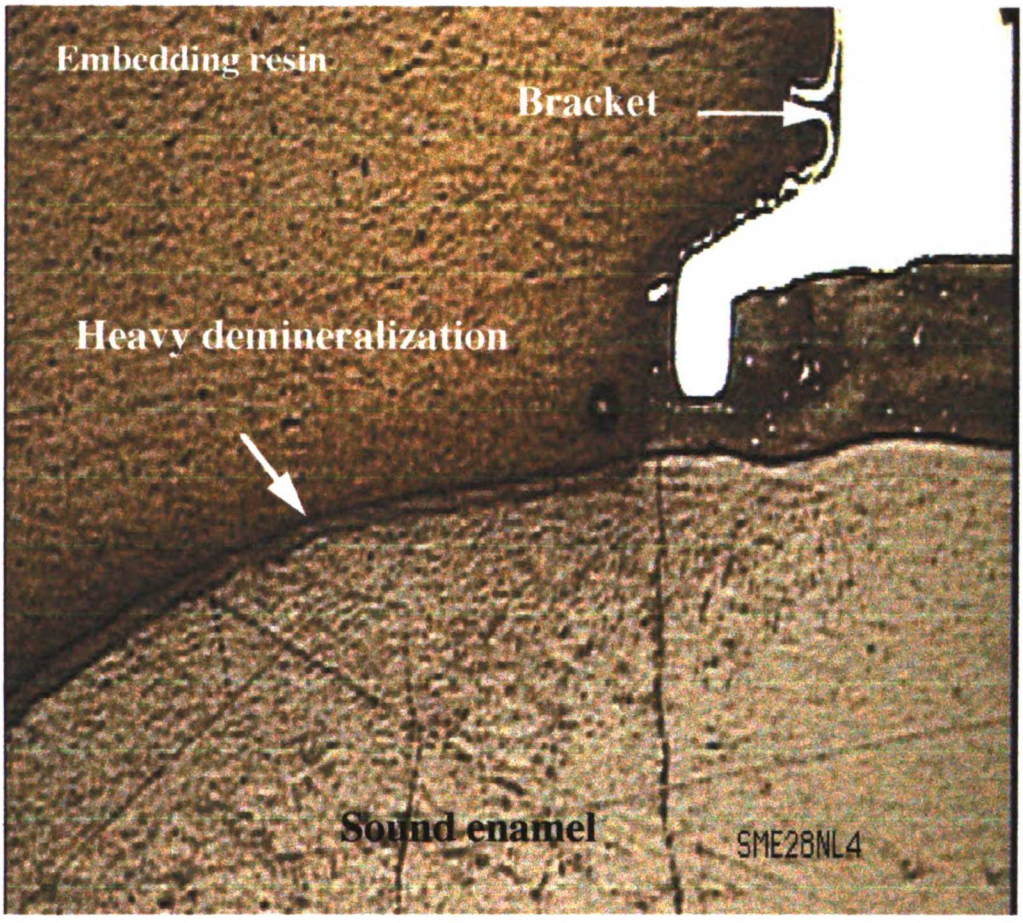


Fig. 12 Non-irradiated enamel showing dark zone of demineralizationon the enamel surface (50x)

Main Study #2 (fluence of 4 J/cm^2): Non-irradiated control enamel after pH-cycling

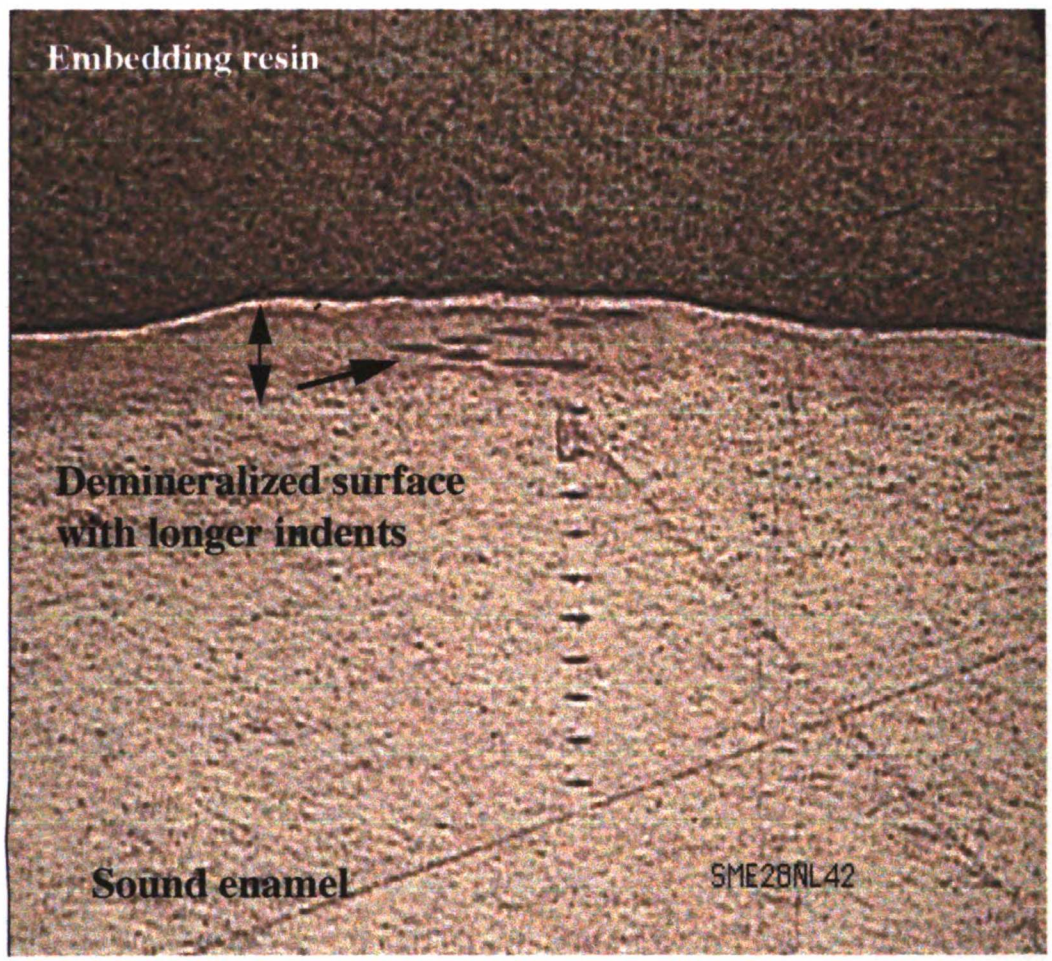


Fig. 13 Non-irradiated enamel showing heavy demineralization (200x)

Main Study #2 (fluence of 4 J/cm^2): Irradiated enamel after pH-cycling

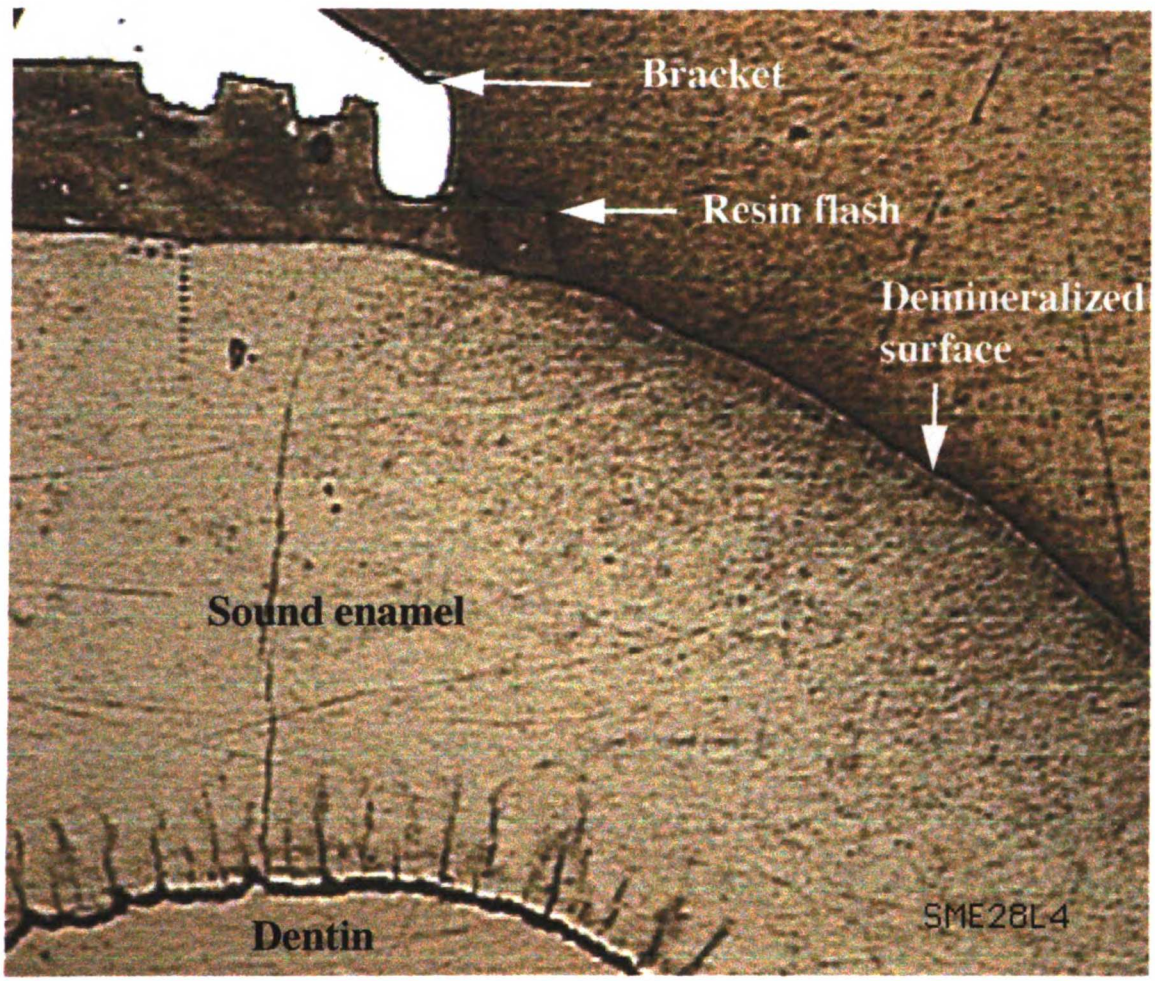


Fig. 14 Irradiated enamel showing a narrow zone of demineralization near the enamel surface. Bracket is partially seen with the resin flash. (50x)

Main Study #2 (fluence of 4 J/cm^2): Irradiated enamel after pH-cycling

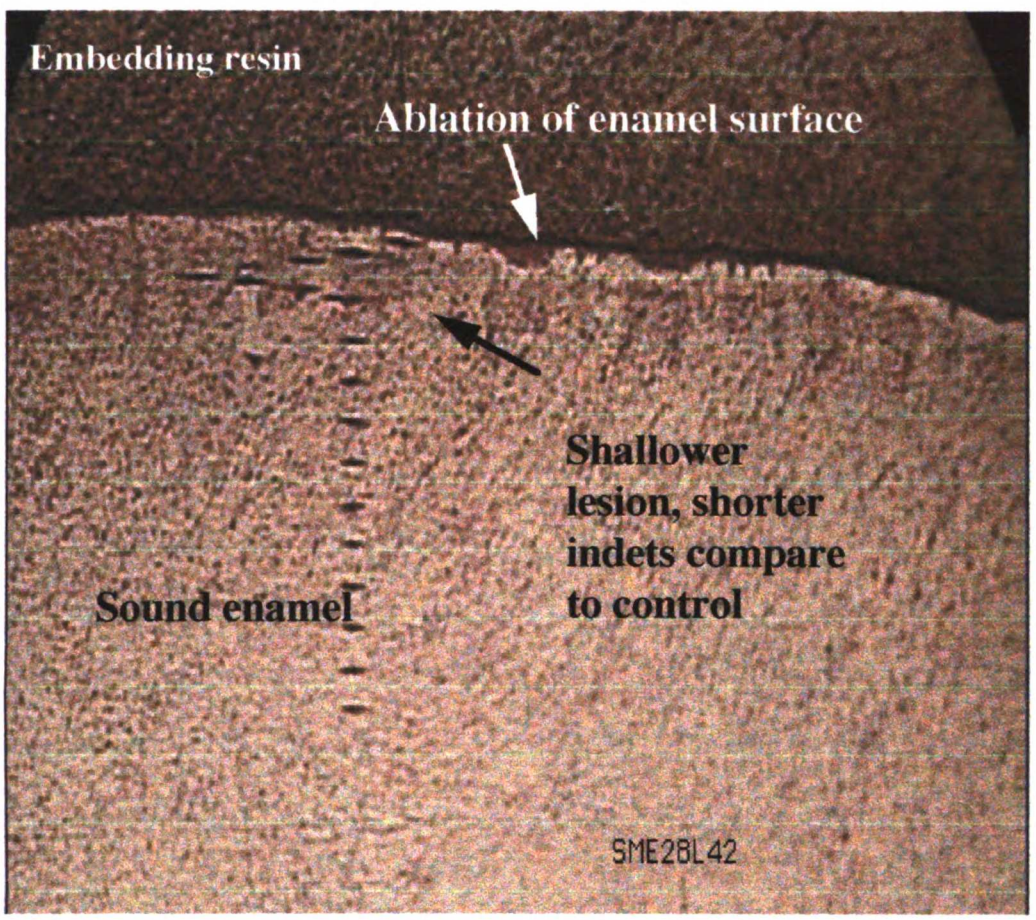


Fig. 15 Irradiated enamel showing shallower lesion than the control,(200x).

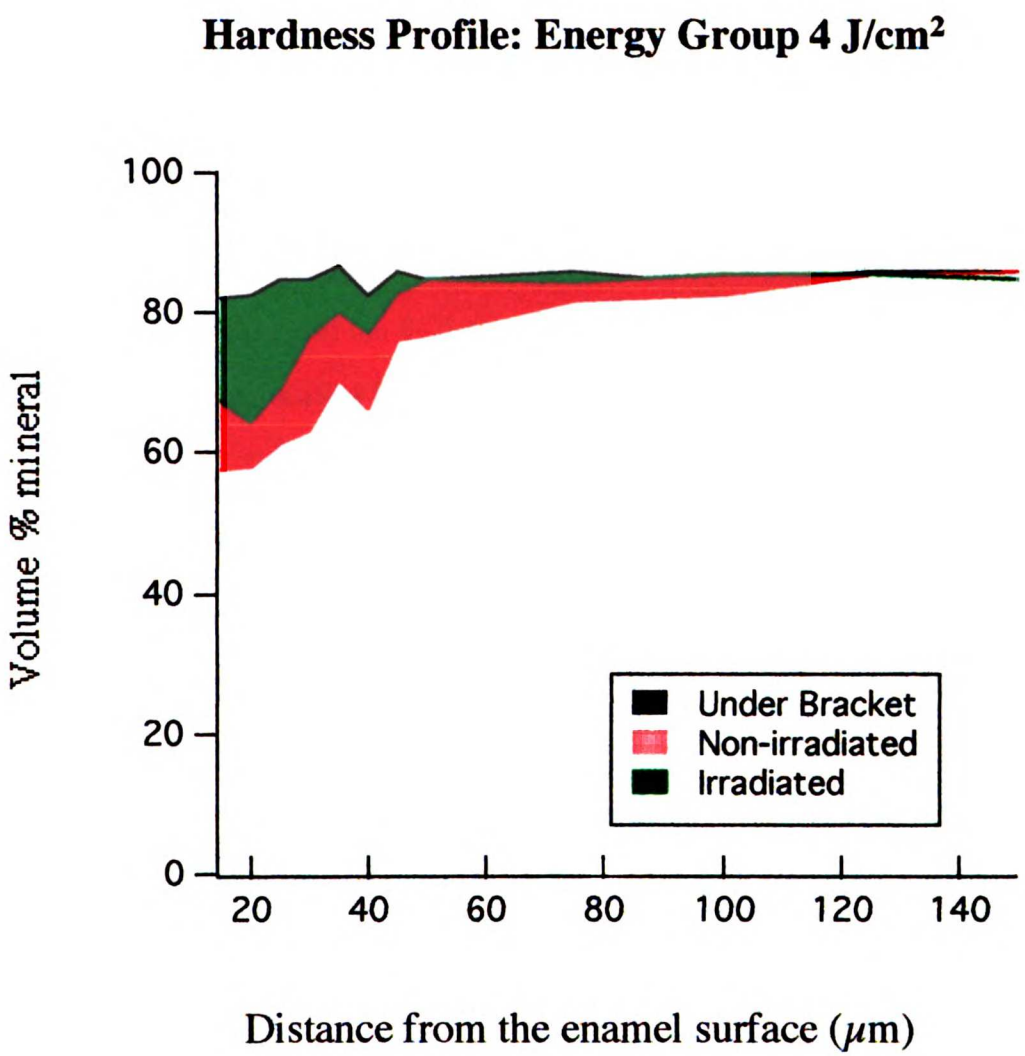


Fig.16 Relative mineral loss of irradiated region is less than non-irradiated region over the whole tested area. Lesion extends to the depth of 120 μm for the control region and to 90 μm for the laser treated region

C.1.4 Main study #3: Glass Ionomer cement and Fluence of 4 J/cm^2

Fig. 17 shows an example of indents in the non-irradiated control enamel low magnification (50X). The same region under higher magnification (200X) is shown in Fig. 18. An example of indents in the laser treated region under low magnification (50X) is shown in Fig. 19 and its higher magnification (200X) in Fig. 20.

Descriptive statistics of ΔZs were performed and shown in Table 6. Although the mean ΔZ value for the non-irradiated side was 13% less than the irradiated side, there was no statistical significant difference among the three regions when compared to each other at $p < 0.05$. The NI side of the 4-GI J/cm^2 group had 83% reduction in mean ΔZ value when compared to main study # 2 control values.

	UB	NI	I
Mean ΔZ	-42.49	137.60	119.63
Std. Dev.	147.59	158.92	113.73
Std.Error	52.18	56.19	40.21
Count	8	8	8
Minimum	-322.20	-69.30	-56.40
Maximum	147.70	427.60	286.10
#Missing	4	4	4

Table 6: Descriptive statistics for main study #3: Glass ionomer and fluence of 4 J/cm^2.

Values are ΔZ (vol% x μm).

Average volume % mineral was plotted against the distance from enamel surface among the three regions of under bracket (UB), non-laser treated (NI), and laser treated (I). The result is displayed in Fig. 21 to show the change in area of relative mineral loss (ΔZ). Almost no decalcification occurred in this group.

Main Study #3(GI- 4 J/cm^2): Non-irradiated control enamel after pH-cycling

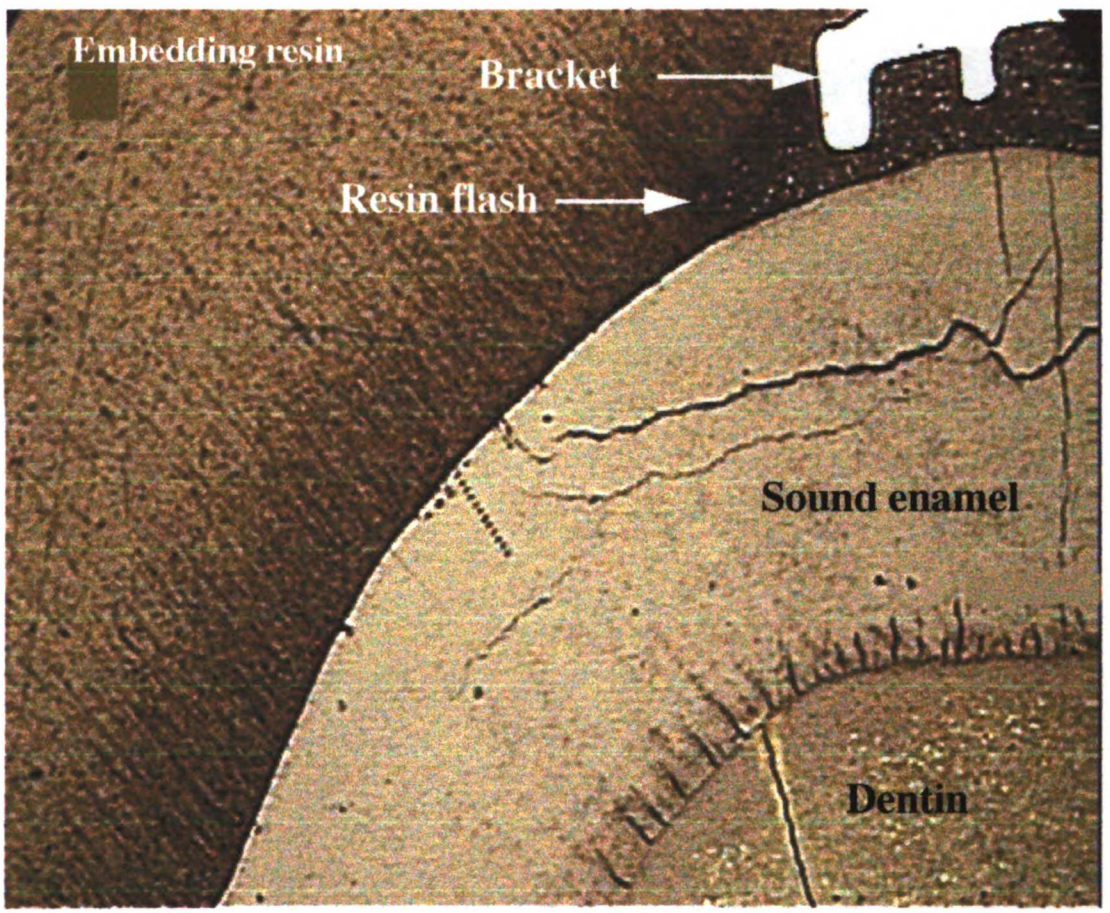


Fig. 17 Non-irradiated enamel, no measurable decalcification is present near the enamel surface (50x)

Main Study #3 (GI- 4 J/cm^2): Non-irradiated control enamel after pH-cycling

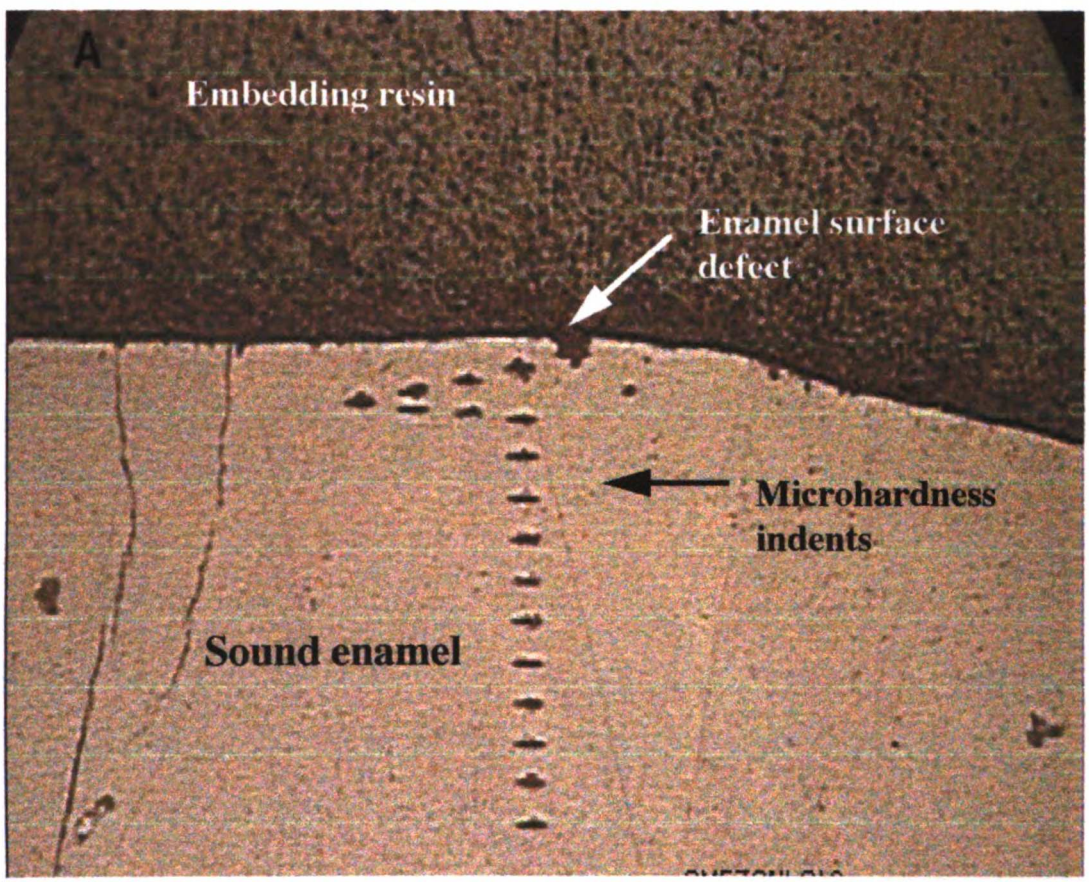


Fig. 18 Non-irradiated enamel, no measurable decalcification is present on the surface (200x)

Main Study #3 (GI- 4 J/cm^2): Irradiated enamel after pH-cycling

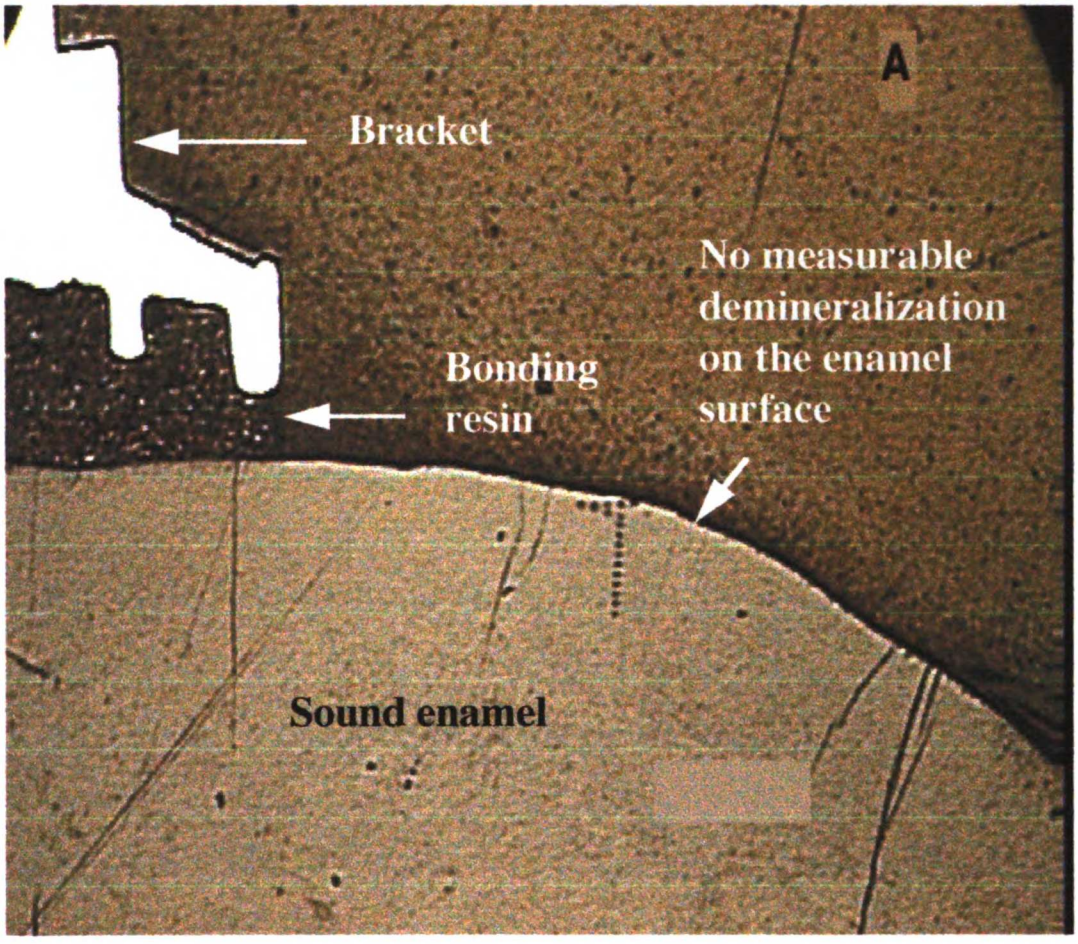


Fig. 19 Irradiated enamel showing no measurable decalcification (50x)

Main Study #3 (GI- 4 J/cm^2): Irradiated enamel after pH-cycling

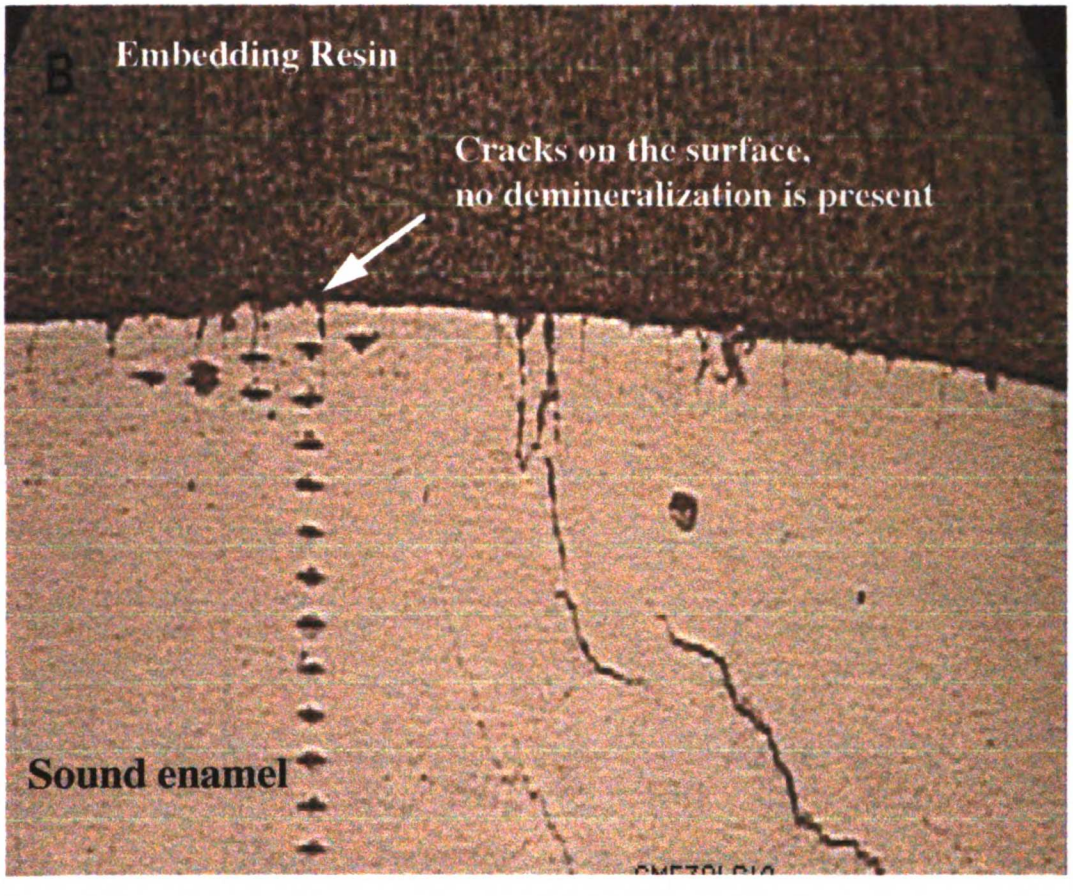
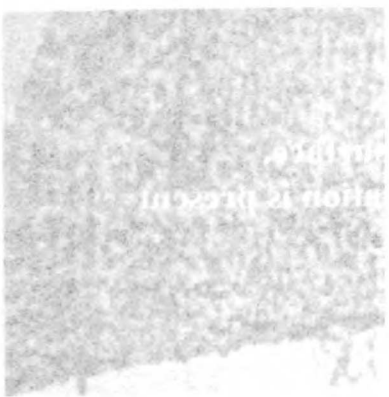


Fig. 20 Irradiated enamel showing no measurable demineralization. Little cracks are present on the enamel surface (200x).



Hardness Profile: Energy Group GI, 4 J/cm^2

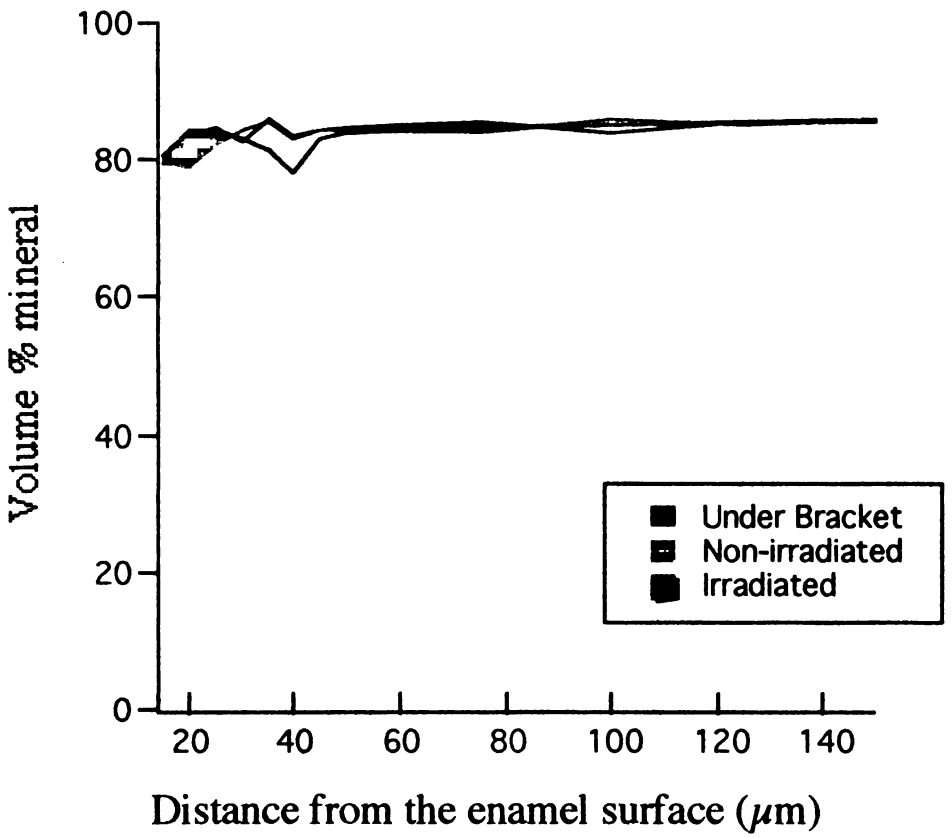


Fig. 21 Total loss of mineral is very small in this group. The irradiated region show more mineral loss in the middle of the lesion depth. This deviation is within the measurement error of +/- 2%.

C.1.5 Main study #4: Fluence of 5 J/cm^2

Fig. 22 shows an example of indents in the non-irradiated control enamel under low magnification (50X). The same region at higher magnification (200X) was shown in Fig. 23. An example of indents in the laser treated region under low magnification (50X) is shown in Fig. 24 and at higher magnification (200X) in Fig. 25.

Descriptive statistics of ΔZs were performed and shown in Table 7. Reduction in mean ΔZ value from non-irradiated to irradiated is 22%. No significant difference was found between the non-irradiated and the irradiated regions at $p < 0.05$. However, both of the non-irradiated and the irradiated regions were statistically different from the under bracket region ($p < 0.05$). The ΔZ value at the non-irradiated region is lower than expected due to difficulties in indent length measurement that is described in the discussion section.

	UB	NI	I
Mean ΔZ	72.36	386.89	300.69
Std. Dev.	74.11	212.32	253.59
Std.Error	21.39	61.29	73.21
Count	12	12	12
Minimum	-31.10	48.40	18.60
Maximum	175.60	877.00	831.00
#Missing	0	0	0

Table 7: Descriptive statistics for main study #4: Fluence of 5 J/cm^2. Values are ΔZ (vol% mineral x μm)

Average volume % mineral was plotted against the distance from the enamel surface among the three regions of under bracket (UB), non-laser treated (NI), and laser treated (I). The result is displayed in Fig. 26 to show the change in area of relative mineral loss (ΔZ). The effect of irradiation to inhibit mineral loss was not as much as with 3 and 4 J/cm^2.

Main Study #4(fluence of 5 J/cm^2): Non-irradiated control enamel after pH-cycling

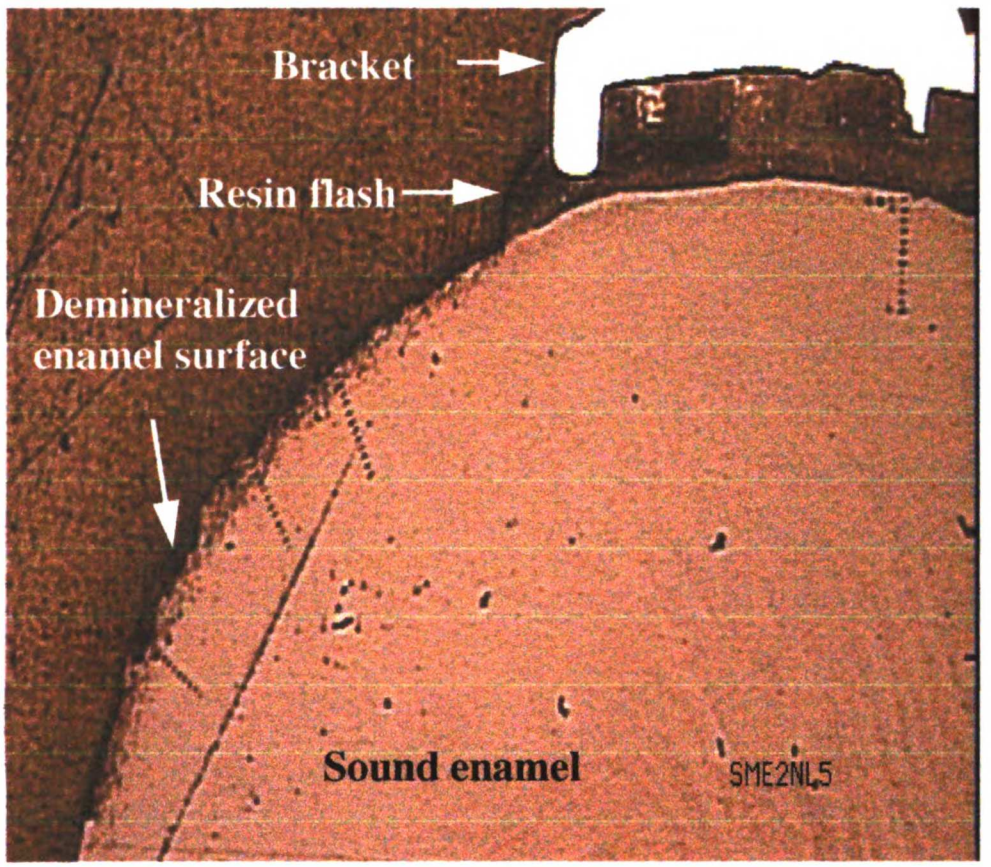


Fig. 22 Non-irradiated enamel showing dark zone of demineralization just below the enamel surface (50x)

Main Study #4 (fluence of 5 J/cm^2): Non-irradiated control enamel after pH-cycling

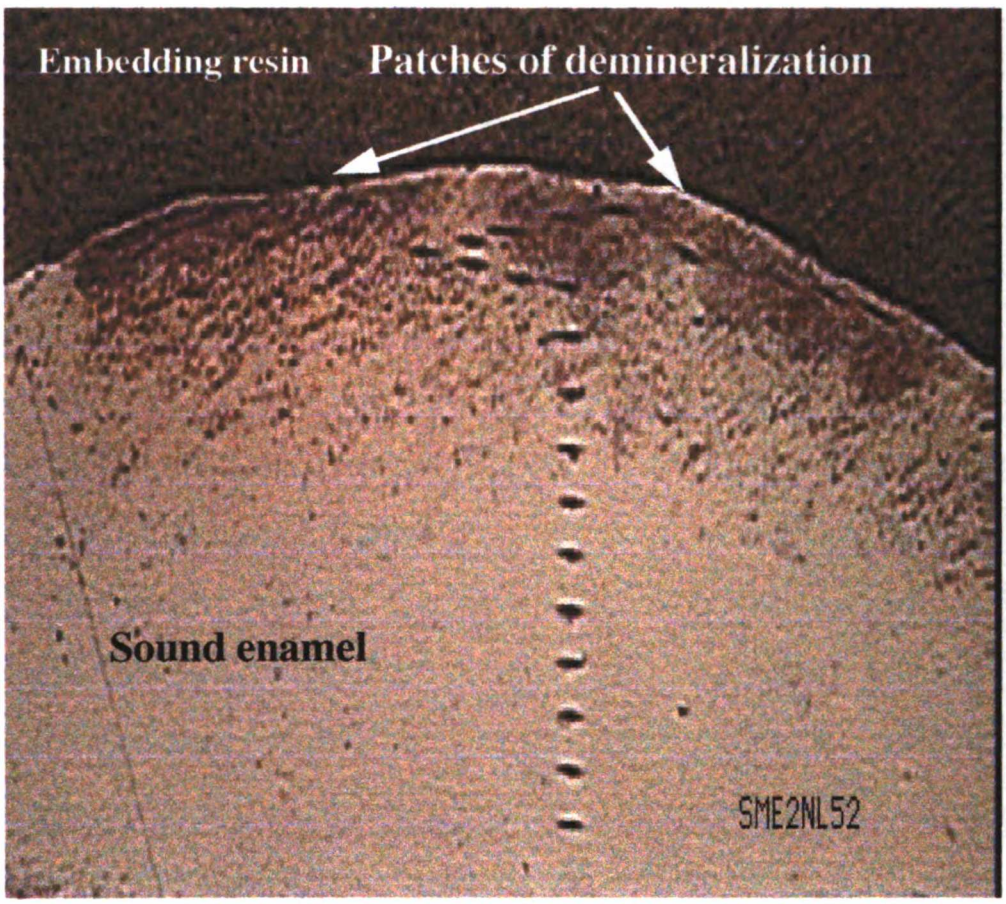


Fig. 23 Non-irradiated enamel showing patches of demineralization just below the enamel surface (200x)


Embedding resin

Main Study #4 (fluence of 5 J/cm^2): Irradiated enamel after pH-cycling

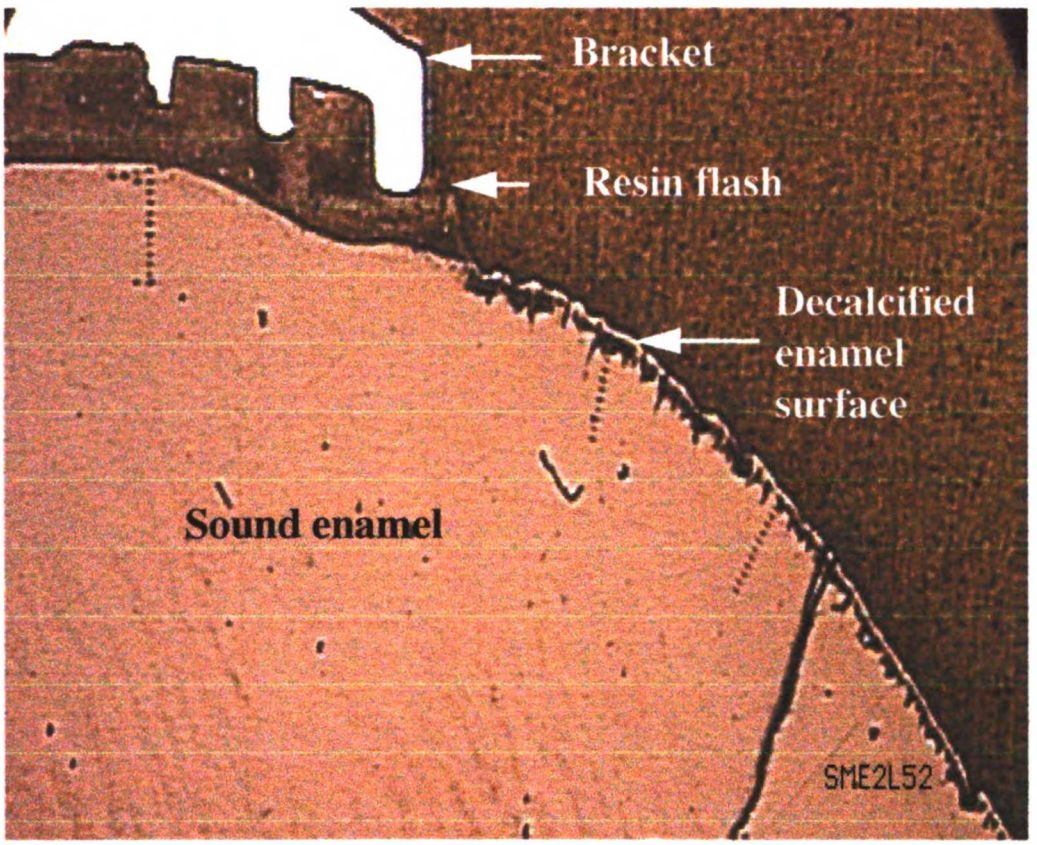


Fig. 24 Irradiated enamel showing dark zone of demineralization just below the enamel surface. This pattern of decalcification is characteristic to this energy group (50x)

Main Study #4(fluence of 5 J/cm^2): Irradiated enamel after pH-cycling

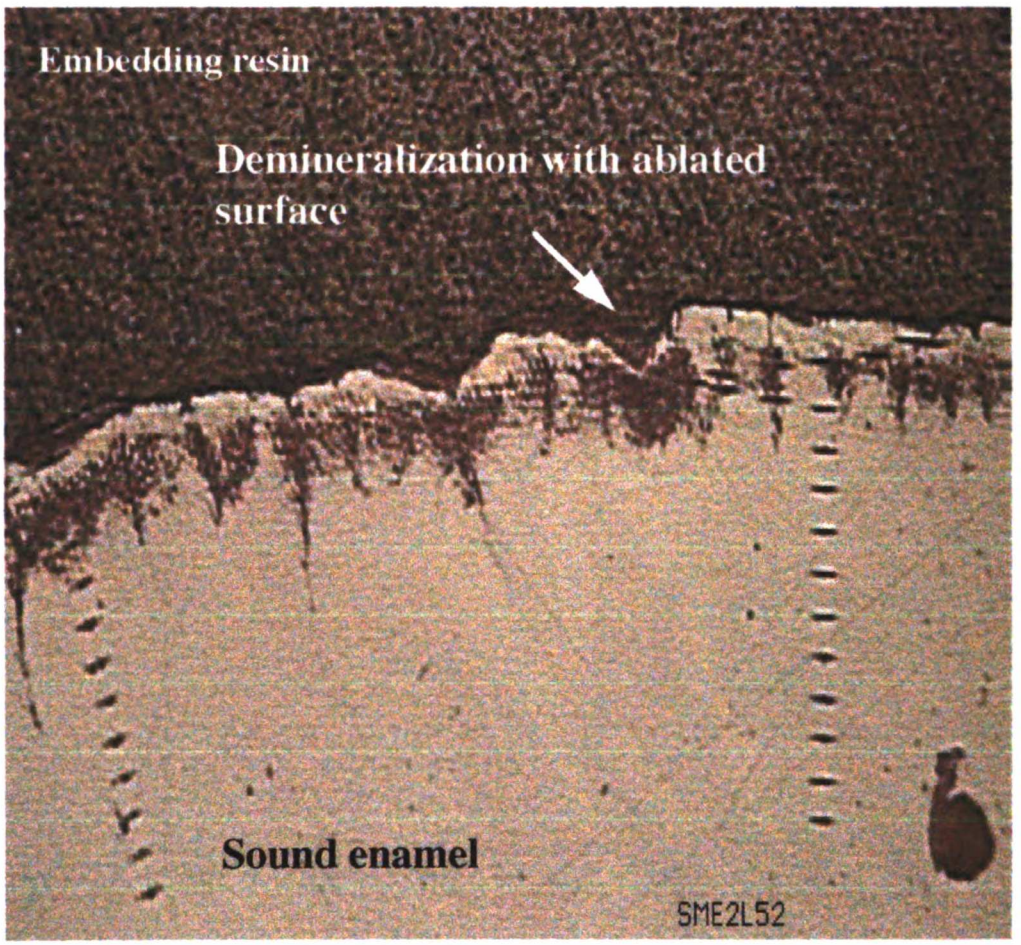


Fig.25. Irradiated enamel showing heavy demineralization just below the ablated enamel surface (200x)

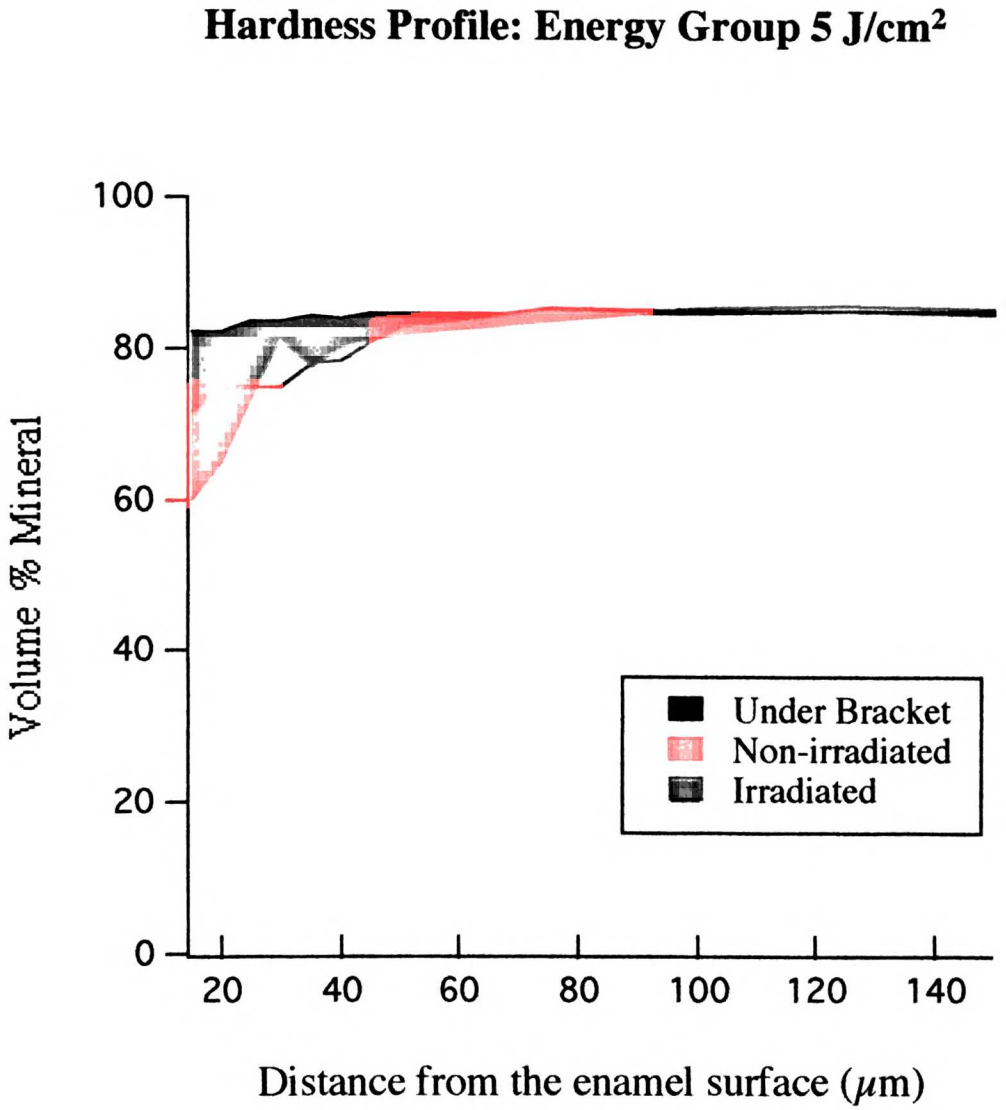


Fig. 26 Overall relative mineral loss of irradiated region is less than the control region. Depth of 35 - 50 μm show more mineral loss than the control region possibly due to measurment error. Lesion is shallower compare to the other groups.

C.2 Thermocouple measurement results:

Tooth A had all four measurements superimposed in figure 27. The average temperature rise in this tooth was 1.5 °C. The linear measurement with the caliper shows a 6 mm distance from the thermocouple to the position of irradiation.

Tooth B had two measurements superimposed in figure 28. An average temperature change of 2.75 °C was measured. The distance of the thermocouple from the irradiated surface in this sample was measured to be 5.0 mm.

Tooth C had three measurements superimposed in figure 29. One measurement was lost. On the average temperature change of 2 °C was observed. The distance of the thermocouple from the irradiated surface was 5.4 mm.

Thermocouple Measure Tooth #1

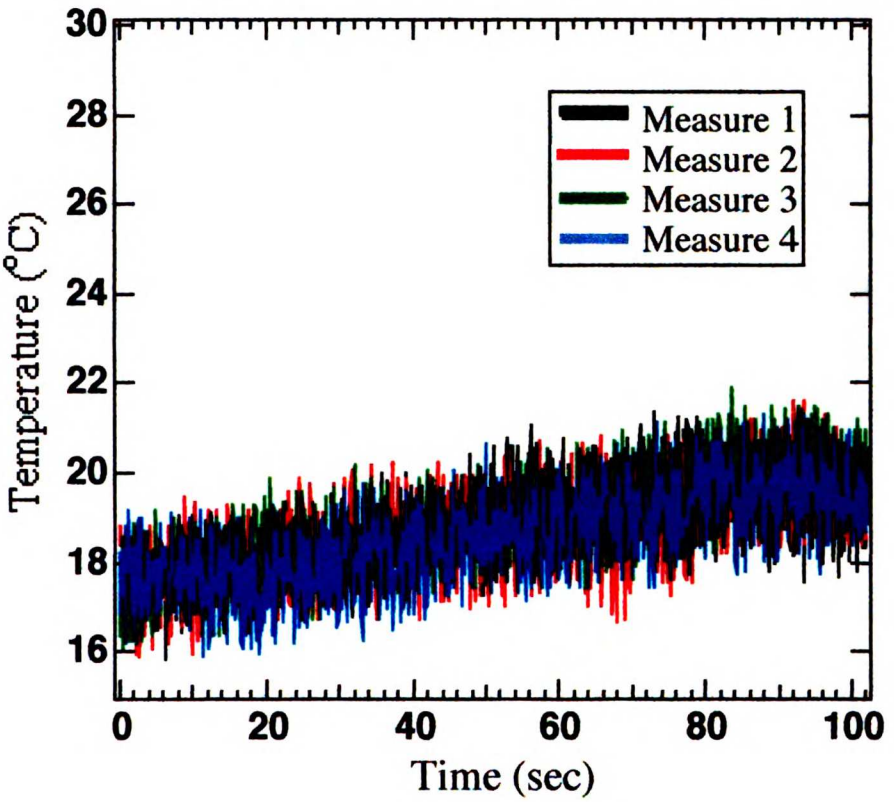
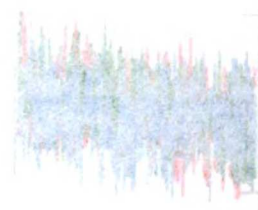


Fig. 27 Four thermocouple measurements are superimposed. Average temperature rise of 1.5° C is calculated.



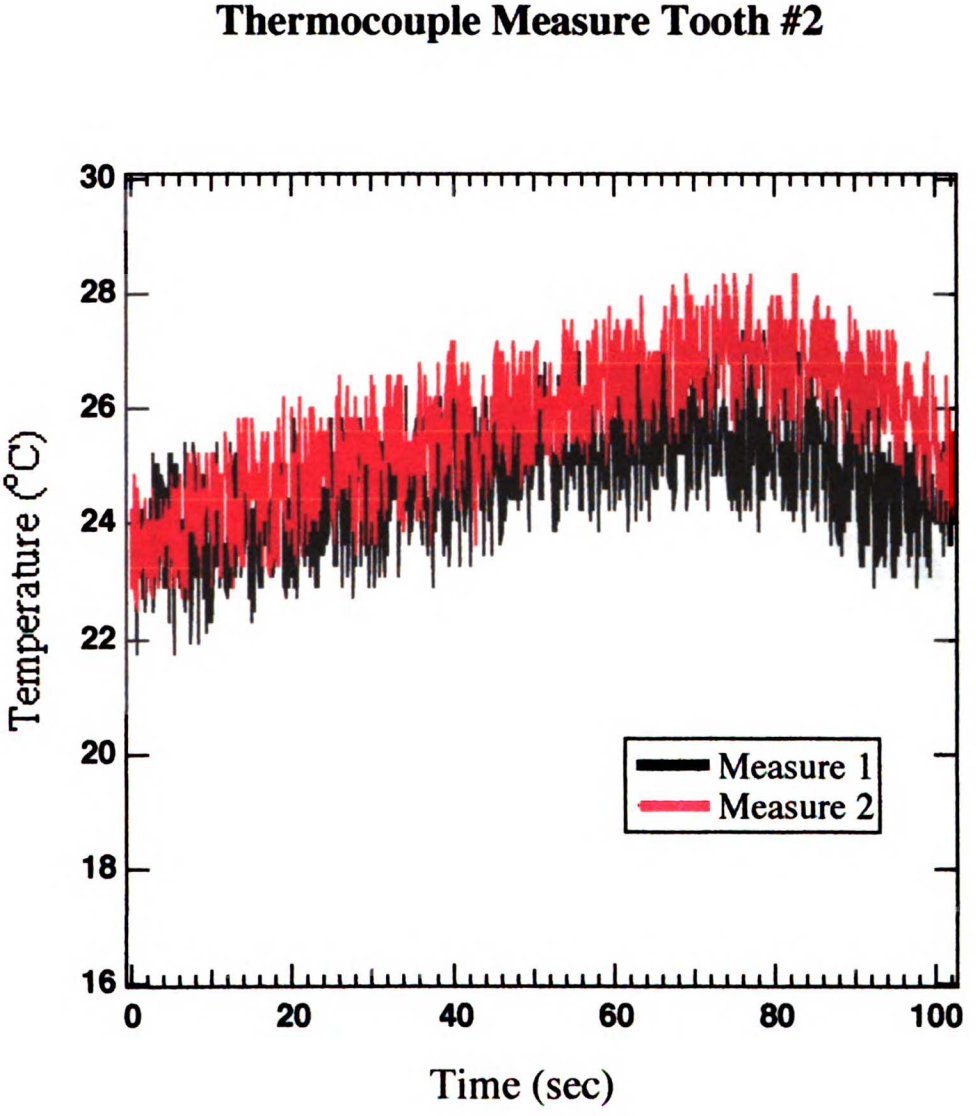


Fig.28 Two thermocouple measurements are superimposed. Average temperature rise of 2.75° C is calculated.

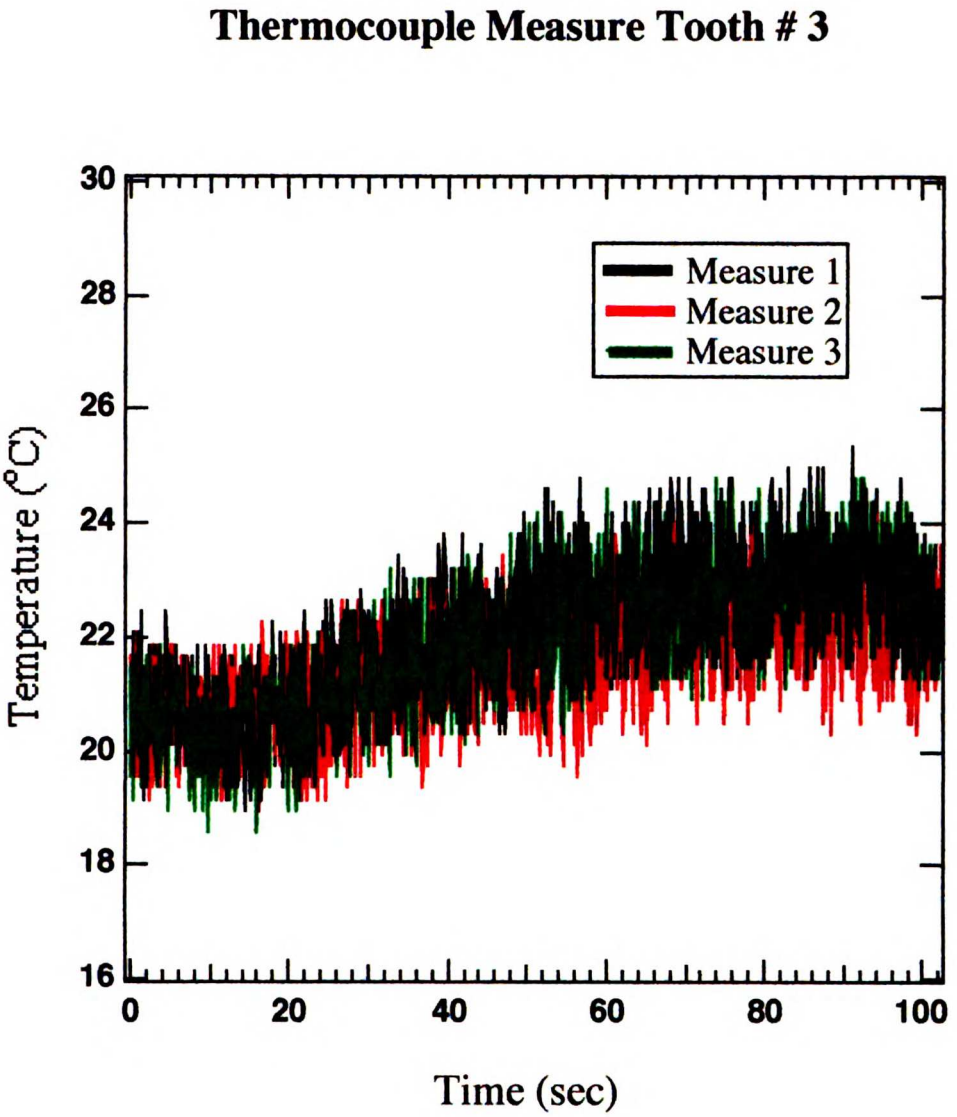


Fig. 29 Three thermocouple measurements are superimposed. Average temperature rise of 2° C is calculated.

D. DISCUSSION

Previous studies have shown that CO_2 laser treatment of the dental enamel surface can inhibit decalcification. The aim of this study was to test the hypothesis that the laser radiation tuned to the absorption maximum of carbonated hydroxyapatite near the 9.6 μm wavelength of the CO_2 laser and pulse duration that is on the order of the thermal relaxation for a 10 μm layer of heated tissue (100 μs) and fluences of 3, 4, or 5 J/cm^2 will optimally inhibit decalcification around orthodontic brackets and protect the surface from formation of white spot lesions without overheating the pulp.

The results from the main studies are summarized in Table 8. The mean ΔZ (vol% mineral x μm) for different regions and their standard deviations (SD) are shown. Laser treated region is compared to the control region for each study and % reduction in the mean ΔZ value and the statistical significane of this comparison ($p<.05$) are reported.

	UB ΔZ (SD)	NI ΔZ (SD)	I ΔZ (SD)	% reduction NI vs. I	P- value
Study #1 Fluence of 3 J/cm²	-49 77	833 470	365 255	56%	< .0001 *
Study #2 Fluence of 4 J/cm²	79 159	1041 568	404 205	51%	< .0001 *
Study #3 Fluence of 4 J/cm², GI	-42 148	138 159	120 113	22%	0.45
Study #4 Fluence of 5 J/cm²	72 74	387 213	301 254	13%	0.9

Tabel 8 Main studies result summary and their statistical significance. ΔZ values are (vol% mineral x μm)

Three regions in each tooth were used for microhardness measurements. Sites under the bracket (UB) were not exposed to the pH cycling solution. Therefore, it was

expected that these enamel sites would remain protected from decalcification. Normal mineralization of 85 volume % was anticipated in microhardness measurement of these areas, unless the enamel was defective or leakage occurred around the brackets. In this experiment the sites under the bracket served as an ultimate control to show the degree of mineralization of each tooth before treatment started. When samples were observed under the microscope, only three of the under bracket regions showed sign of leakage or defective enamel. No significant differences were found in comparison of the mean ΔZ values for under-bracket regions of energy groups of 3, 4, 4-GI, and 5 J/cm^2 (refer to Table 10).

Non-irradiated regions and irradiated regions were simultaneously exposed to the pH cycling solution. Decalcification was expected to be seen in these areas, with the irradiated sites having less decalcification than the non-irradiated sites. Non-irradiated sites served as a control for the irradiated sites on each individual tooth.

The laser side of all energy groups had a peculiar characteristic. Small cracks were present perpendicular to the enamel surface. This effect on enamel can be seen in Figs. 9, 10, 19, 20, 25, and 26. Cracks on enamel can cause subsurface demineralization to occur by allowing penetration of solution inside enamel. Another important observation associated with the laser treatment was the presence of areas of ablation on the enamel surface at higher fluences. Higher energy lasers have been shown to create ablation. Irradiated regions of all the teeth in energy group 5 J/cm^2 showed a similar pattern of enamel ablation. This distinct pattern is displayed in Figs. 25 and 26. In Fig. 26, areas of ablation have subsurface decalcification associated with them. Smaller and more scattered ablations were seen in energy group 4 J/cm^2. This is shown in Fig. 15 with ablated spots very close to the indented areas.

These ablation regions are most likely related to "hot spot" in the beam. The enamel defects, cracking and ablation, caused by laser treatment is due to the variation of the beam intensity over the beam diameter. Cracks and ablations are generated at the locations of the

beam maximum intensities and they can be prevented by making the beam energy more uniform across the beam diameter.

Non-irradiated sites also showed characteristic patterns. Either decalcification was seen as a uniform dark band over the enamel surface (Fig. 8) or patches of dark areas (Fig. 23). No measurable decalcification was observed in the non-irradiated sites of the 4-GI J/cm^2 group.

Energy group 4-GI J/cm^2 did not show any significant difference between the mean ΔZ values of irradiated and non-irradiated regions (refer to Table 8). Also, no significant difference was present when these two regions were compared with under-bracket regions of other groups (refer to Table 10). These two sites did not manifest any significant difference to the under-bracket region of their energy group and other energy groups. This result indicates that minimum decalcification occurred in the enamel of 4-GI J/cm^2 energy group with or without radiation. This result is shown in Figs. 17 and 19. The one difference that this sample had compared with the samples for other energy groups was the bonding medium. Glass ionomer bonding material was used in this group. GC Fuji ORTHO is a self-cure resin that is reinforced by glass ionomer bonding material. This cement has been shown to release fluoride over a long period of time. The disadvantage of cement is the higher possibility of bond failure. As a caries preventive matter, use of this cement is very effective in inhibiting decalcification. The manufacturer claims that GC can release up to 5 μg/cm^2 fluoride over a year. It is not clear if the fluoride release continues after one year and whether the concentration of fluoride over a longer time remains at an effective level. When the control regions of the main study #2 and #3 were compared, 83% inhibition of decalcification was observed and comparison of the irradiated regions showed 70.3% inhibition of decalcification. In the present experiments the fluoride release was sufficient to prevent demineralization completely even without laser irradiation. With the scope of this study it is not possible to determine the synergistic effect of laser treatment and the fluoride since the sample size is not sufficient.

In comparing mean ΔZ values of the three regions, significant results were obtained for energy groups 3 and 4 J/cm^2. The results were ambiguous for group 5 J/cm^2. Energy group 5 J/cm^2 did not exhibit the expected amount of decalcification in the non-irradiated sites (refer to Table 8). One explanation is that indent placement started with this group. Indents were placed in the samples of this group by using the indentor microscope. Unfortunately, a few rows of indents were placed before length measurement of these indents started. It turned out that the tip of the indentor was not clean and the created "fuzzy" indents were unmeasurable. This is shown in Fig. 25 where the left indents are unreadable. Main areas of decalcification around the brackets were rendered unmeasurable due to this technical difficulty which resulted in low readings of mean ΔZ values. It was observed that decalcification occurred in the irradiated sites of samples in this energy group mainly around the ablated enamel surfaces. This is not reflected in the mean ΔZ values calculated for this group. An explanation for this discrepancy is that indents were placed at the areas that ablation did not occur. Ablated areas contained uneven surfaces of enamel that prevented a scatter pattern of indents from being placed accurately. Therefore, the relative mineral loss of the irradiated sites in this energy group is under estimated. For these reasons, the statistical analysis shows no significant difference between non-irradiated and irradiated sites of this energy group (refer to Table 8). However, there is a significant difference to the under bracket region indicating decalcification occurred (refer to Table 10).

Energy groups 3 and 4 J/cm^2 were the most effective laser conditions with 56% and 51% reduction of decalcification respectively. Statistically significant differences ($p < .0001$) were observed when the mean ΔZ values of the non-irradiated sites and irradiated sites were compared within the group. This result once again proves that specific conditions of the laser can change the enamel to make it more resistant to decalcification. The amount of relative mineral loss in non-irradiated regions of the two energy groups

when compared, showed no statistically significant difference and comparison of the irradiated sites of the two groups showed no statistically significant difference (refer to Table 10). It seems that both fluences were equally effective at inhibiting decalcification on the enamel surface. However, with the fluence of 3 J/cm^2 less surface enamel defect was observed.

Featherstone et al., 1997, advanced our understanding of the mechanism of demineralization inhibition by laser treatment. They showed that loss of carbonate (CO_3) from the enamel surface was fluence and wavelength dependent. Their study demonstrated that total loss of carbonate occurs at wavelength of 9.6 μm and fluence of 4 J/cm^2. It was concluded by this study that irradiation of enamel with specific parameters alters the chemical composition of enamel crystals, causing decomposition of the CO_3 component and therefore, reducing the reactivity of enamel (Featherstone et al., 1997). While irradiation with fluence of 4 J/cm^2 was quite effective at removing the carbonate component, the irradiation with fluence of 3 J/cm^2 only caused partial loss of carbonate from the enamel surface. In order to explain the effectiveness of fluence of 3 J/cm^2 in demineralization inhibition, it is our thought that other structural changes or chemical compositions besides carbonate are involved in caries inhibition when the enamel surface is heated to the temperature of 550 °C.

The length of time that it takes to irradiate the enamel surface is an issue that has direct clinical significance. The laser used in this study had a beam size of 1 mm and pulse repetition of 10 Hz with 25 pulses per spot. Hence, it takes 2.5 seconds to irradiate a spot that is 1 mm in diameter. If the irradiated spots are overlapping to provide uniform coverage of the surface e.g. 0.5 mm in this study, then it takes longer to irradiate the surface and the time can be calculate from the following formula :

2.5 seconds x (2 x distance irradiated in mm - 1)

It is also necessary to take into account the time that it takes for the operator to move from one spot to the next.

Resin flash around the brackets is a source of plaque accumulation intraorally. Clinicians spend a good time during bracket placement to clean the resin flash. It was an attempt of this experiment to look at the resin flash removal with the same conditions of laser treatment previously discussed. The result showed that the resin flash remained intact even after laser treatment. This observation can be seen in Figs. 14 and 24. It is possible that a higher laser fluence is required for this removal.

The results from the temperature measurements were not conclusive. Three teeth were irradiated with 3 J/cm^2 on a spot located at the middle portion of the buccal groove. The distance between the buccal groove (above the bracket) was measured to the mesio-buccal horn of the pulp where the thermocouple was placed. It was shown that the temperature rose from 1.5 to 2.75°C when the distance varied from 5-6 mm. This temperature is within the acceptable range not to cause any harm to the pulp (Zach and Cohen, 1977). However, this result is inconclusive since we do not know if this is the maximum temperature rise in the pulp. To get the maximum temperature rise, the thermocouple needed to be co-linear with the irradiated region on the surface at the shortest distance possible. The average shortest distance was measured on the tested teeth after they were cut and it was 4 mm. Therefore, co-linealizing the irradiation spot on the surface with the thermocouple is a task that needs further investigation.

The significance of this research is directly related to the ultimate clinical utility. For clinical applications, the fracture and wear resistance of laser modified enamel must be thoroughly investigated before this treatment is acceptable for clinical use, because laser treatments may introduce stresses, cracking, and ablation that reduce the structural integrity of dental enamel. The reflectance energy and its interaction with the peripheral tissue, especially soft tissue, must be investigated before this laser can be applied clinically.

Researchers are exploring other possibilities for laser use in the clinical setting. The treatment of carious lesions, pits, and fissures, and occlusal surfaces, etching of enamel, removal of composite after debracketing, increasing retention of bonding resin, are a few examples. If the laser technology that has been shown to be effective in the laboratory becomes available at a reasonable cost and the results are substantiated clinically, there will indeed be a future for the clinical application of lasers in preventive dentistry.

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F. APPENDIX

The mean ΔZ values for all three regions, under bracket (UB), non-laser treated (NI) and laser treated (I), in four energy groups of 3, 4, 4-GI, 5 J/cm^2 were ranked and compared to one another. Fifteen cases were omitted due to unequal sample size of four energy groups. The results are summarized in the following tables:

	DF	Sum of squares	Mean squares	F-value	P-value
Energy Groups	11	13679945.93	1243631.448	16.363	<.0001
Residual	117	8892270.1	76002.309		

Model 11 estimate of between component variance: 108846.784

Table 8: Anova table for energy groups

	Mean Diff.	Crit. Diff	P-value	Significance
UB-3, NL-3	-882.48	222.90	<.0001	S
UB-3, L-3	-414.45	222.90	0.00	S
UB-3, UB-4	-127.31	227.91	0.27	
UB-3, NL-4	-1089.40	222.91	<.0001	S
UB-3, L-4	-452.86	227.91	0.00	S
UB-3, UB-5	-121.59	222.90	0.28	
UB-3, NL-5	-436.13	222.90	0.00	S
UB-3, L-5	-349.93	222.90	0.00	S
UB-3, UB-GI	-6745.00	249.21	0.96	
UB-3, NL-GI	-186.83	249.21	0.14	
UB-3, L-GI	-168.86	249.21	0.18	
NL-3, L-3	468.03	222.90	<.0001	S

NL-3, UB-4	755.18	227.91	<.0001	S
NL-3, NL-4	-206.91	227.91	0.07	
NL-3, L-4	429.62	227.91	0.00	S
NL-3, UB-5	760.89	222.90	<.0001	S
NL-3, NL-5	446.36	222.90	0.00	S
NL-3, L-5	532.56	222.90	<.0001	S
NL-3, UB-GI	875.74	249.21	<.0001	S
NL-3, NL-GI	695.65	249.21	<.0001	S
NL-3, L-GI	713.63	249.21	<.0001	S
L-3, UB-4	287.14	227.91	0.01	S
L-3, NL-4	-674.95	227.91	<.0001	S
L-3, L-4	-38.41	227.91	0.74	
L-3, UB-5	292.86	222.90	0.01	S
L-3, NL-5	-21.68	222.90	0.85	
L-3, L-5	64.53	222.90	0.57	
L-3, UB-GI	407.71	249.21	0.00	S
L-3, NL-GI	227.62	249.21	0.07	
L-3, L-GI	245.59	249.21	0.05	
UB-4, NL-4	-962.09	232.81	<.0001	S
UB-4, L-4	-325.56	232.81	0.01	S
UB-4, UB-5	5.71	227.91	0.96	
UB-4, NL-5	-308.82	227.91	0.01	S
UB-4, L-5	-222.62	227.91	0.06	
UB-4, UB-GI	120.56	253.79	0.35	
UB-4, NL-GI	-59.53	253.79	0.66	

UB-4, L-GI	-41.55	253.70	0.75	
NL-4, L-4	636.54	232.81	<.0001	S
NL-4, UB-5	967.81	227.91	<.0001	S
NL-4, NL-5	653.27	227.91	<.0001	S
NL-4, L-5	739.47	227.91	<.0001	S
NL-4, UB-GI	1082.65	253.70	<.0001	S
NL-4, NL-GI	902.56	253.70	<.0001	S
NL-4, L-GI	920.54	253.70	<.0001	S
L-4, UB-5	331.27	227.91	0.00	S
L-4, NL-5	16.74	227.91	0.88	
L-4, L-5	102.94	227.91	0.37	
L-4, UB-GI	446.12	253.70	0.00	S
L-4, NL-GI	266.03	253.70	0.04	S
L-4, L-GI	284.00	253.70	0.03	S
UB-5, NL-5	-314.53	222.90	0.01	S
UB-5, L-5	-228.33	222.90	0.04	S
UB-5, UB-GI	114.85	249.21	0.36	
UB-5, NL-GI	-65.24	249.21	0.61	
UB-5, L-GI	-47.27	249.21	0.71	
NL-5, L-5	86.20	222.90	0.45	
NL-5, UB-GI	429.38	249.21	0.00	S
NL-5, NL-GI	249.29	249.21	0.05	S
NL-5, L-GI	267.27	249.21	0.04	S
L-5, UB-GI	343.18	249.21	0.01	S
L-5, NL-GI	163.09	249.21	0.20	

L-5, L-GI	181.07	249.21	0.15	
UB-GI, NL-GI	-180.09	272.99	0.19	
UB-GI, L-GI	-162.11	272.99	0.24	
NL-GI, L-GI	17.98	272.99	0.90	

Table 9: Fisher's PLSD for energy groups (Significance level: 5%)