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Modification of dental enamel reactivity by a CO_2 tea laser

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MODIFICATION OF DENTAL ENAMEL REACTIVITY BY A CO_2 TEA LASER

by

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THESIS

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ABSTRACT

Many studies have shown that pulsed CO_2 laser irradiation can be used to induce modification of dental enamel by thermal decomposition of the apatite mineral, resulting in reduced reactivity of this tissue to subsequent acid challenge. Reduction in acid reactivity, which in the laboratory is manifested as reduced rates of initial surface acid dissolution and reduced sizes of artificial caries-like lesions, has been closely associated with loss of carbonate from the enamel crystal and transformation to a less substituted and thereby less acid susceptible mineral. In this study, a TEA CO_2 laser with wavelength well matched to the peak absorption of the dental mineral and pulse duration commensurate with the thermal relaxation time of enamel was evaluated for its caries preventive potential on dental hard tissue. For this purpose, bovine enamel surfaces were treated either at 9.6 μm or at 10.6 μm with 5 pulses per spot, of 2 μs pulse duration, delivered at 1 Hz repetition rate, with incident fluences 0-1 J/cm^2 and 0-4 J/cm^2 respectively. The laser-induced surface changes were evaluated by optical microscopy, FTIR spectroscopy, and surface acid-dissolution experiments. At the highly absorbed 9.6 μm wavelength, where the selected pulse duration matched closely the thermal relaxation time of tissue (~1 μs), effective heating of enamel to the temperatures required for surface modification took place at absorbed fluences of less than 0.5 J/cm^2. The less efficiently absorbed 10.6 μm wavelength required absorbed energies several times higher to produce similar results (~3-4 J/cm^2). The reduction in the relative surface dissolution rates closely paralleled the reduction in carbonate band intensities in accordance with previous reports using longer pulses (100 μs). At both wavelengths, short pulses seemed to lower the

energy requirements for effects comparable to longer pulses, with more dramatic reduction evident at 9.6 μm. These results indicate that a TEA CO_2 laser at 9.6 μm, with a 2 μs pulse duration, may be a practical and efficient laser system for caries preventive enamel surface modification with minimal heat transfer to the interior of the tooth and therefore, minimal risk for pulpal necrosis.

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A. INTRODUCTION

For the last two decades, a marked decline in the prevalence of dental caries in children has been noted in most countries of the western world. In USA, national studies conducted over the past 30 years show that this decline is not equal across the population of children aged 5-17 years and that the prevalence of dental caries demonstrates an interesting age-group specificity.[1-6] For example, by the late 1980s about 75% of children in the 5-11 years age group were caries free, but 70% of children aged 12-17 years had caries. In addition, recent studies indicate that in the 1990s the decline in dental caries may have reached a plateau.[6] In adults, dental caries seems to continue being a major problem. A recent US survey has reported that 94% of all dentate adults aged 18 years and above had evidence of treated or untreated coronal caries.[7] These findings indicate the need for an improved approach to prevention and therapy incorporating individual risk assessment and specific targeting of high-risk persons and populations.

At present, the processes involved in the mechanism of dental caries are well understood and a number of established methods exist for caries prevention and early intervention. In addition to these methods, some innovative and exciting new techniques have shown research successes and great potential for use in caries prevention in the near future. These include chairside molecular probes for assessment of bacterial challenge, computerized risk assessment programs, caries immunization by genetically engineered IgAs, early diagnostic methods like optical coherence tomography and fluorescence, and lasers for conservative caries removal and/or caries prevention.

Early studies in the 1960s and 1970s demonstrated the potential of lasers to increase the acid resistance and alter the crystallographic properties of dental enamel *in vitro*, despite their empirical experimental approach and their irradiation conditions, which were impractical for clinical use.[8-12] In the 1980s and 1990s, laser studies for dental hard tissue applications adopted a more methodical approach with emphasis on understanding the optical and thermal properties of laser-tissue interactions. Featherstone, Fried, and coworkers, in particular, are among the pioneer investigators striving during the last 20 years to establish a sound scientific basis for the effects of lasers on dental hard tissues. Their extensive experimentation has led to a range of irradiation conditions that seem to have the highest potential for caries prevention purposes.[13-20] These include the carbon dioxide wavelengths, especially at 9.3 and 9.6 μm, as the primary choice for caries inhibition due to their extremely high absorption by the dental mineral, and a pulsed mode with pulse durations close to the thermal relaxation time of dental enamel. The ultimate goal of these experimental efforts has been to identify a specific set of irradiation conditions, which can most effectively and efficiently modify the dental mineral to a less soluble form with the lowest possible energy expenditure.

The present study falls within the realm of those researchers' overall objective to optimize the laser parameters most suitable for caries preventive treatments in the clinical setting. The particular choice of irradiation conditions was mainly driven by recent accurate estimates of absorption coefficients and thermal relaxation times of enamel for each of the four major CO_2 laser wavelengths.[15] These estimates placed the thermal relaxation time of deposited energy in dental enamel after irradiation with laser light of

9.6 μm wavelength at 1 μs. As a consequence, an interest was generated to test the potential of CO_2 laser systems capable of such short pulse durations to beneficially modify the enamel surface and enhance its acid resistance.

The specific hypothesis to be tested was that the highly absorbed 9.6 μm laser irradiation, delivered in 5 short (1-4 μs) pulses per spot (very similar to the thermal relaxation time of enamel for this wavelength) will:

a) Need less energy to cause compositional and morphological surface modification and increased acid resistance comparable to the ones caused by longer (100 μs) pulses previously tested (i.e., at lower incident and absorbed fluences), and

b) Need much lower absorbed fluences to achieve compositional, morphological, and acid reactivity changes comparable to the ones caused by short, 10.6 μm pulses.

If this hypothesis were to prove true, it would open the path for highly efficient and very safe (in terms of pulpal damage) laser treatment modalities for caries prevention.

B. LITERATURE REVIEW

B.1 LASERS IN DENTISTRY – HISTORICAL

Maiman (Hughes Research Laboratories) built the first working laser in 1960.[21] This was a pulsed ruby instrument, which emitted light of 0.694 μm wavelength in the visible region of the electromagnetic spectrum. Since then, a number of researchers documented the ability of different types of lasers to cut, coagulate, ablate, and vaporize biologic tissues, including dental hard and soft tissues. In the early years, synthetic ruby was the only material used routinely as the active medium in lasers. Therefore, most of the initial studies used the ruby laser to explore laser light interaction with enamel and dentin.[8,9] Later on, with the advent of lasers making use of different active media, other wavelengths were investigated.

In 1972, the U.S. Food and Drug Administration (FDA) cleared for marketing the first laser for intraoral use. This was a carbon dioxide (CO_2) laser, which emitted light of 10.6 μm wavelength in the mid-infrared region of the electromagnetic spectrum. For nearly two decades, intraoral use of CO_2 lasers was confined primarily to otolaryngologists, oral surgeons, and some periodontists. In 1990, the FDA approved the first laser designed specifically for general dentistry and intraoral soft tissue surgery by Myers et al.[22] This was a neodymium: yttrium-aluminum-garnet (Nd:YAG) laser, which emitted light of 1.0646 μm wavelength in the near infrared region of the electromagnetic spectrum. Since then, other FDA dental laser marketing clearances followed. The first laser for dental hard tissue applications, namely caries removal and cavity preparation,

was cleared for marketing in 1997. This was an erbium: yttrium-aluminum-garnet (Er:YAG) laser, which emitted light of 2.94 μm wavelength in the mid-infrared region of the electromagnetic spectrum, and was designed by Premier Laser Systems.[23]

Today, several laser wavelengths are being used clinically in dental practices in the United States. The soft tissue surgical lasers are the most widespread. The most prevalent soft tissue lasers are the Nd:YAG, CO_2, and diode lasers although the argon, Nd:YAP, Ho:YAG, Er:YAG, and Er:YSGG are also used for ablation, incision, excision, and coagulation of soft tissues. Diode lasers are used to perform pulpotomies as an adjunct to root canal treatment. Er:YAG and Er:YSGG are used for dental hard tissue applications that include caries removal, cavity preparation, and enamel surface modification. The argon laser is used for composite polymerization.

In addition to the above FDA-cleared clinical uses of lasers, other dental applications and other types of lasers are currently the subjects of vigorous research. Some examples include:

- CO_2, argon, and Nd:YAG lasers for caries prevention by rendering enamel less susceptible to decay.
- Excimer lasers for caries removal, enamel, dentin, and bone ablation, and endodontics.
- Nd:YAP laser for caries ablation and endodontics.
- CO_2, Nd:YAG, Ho:YAG, and argon lasers for desensitization of hypersensitive dentin.
- Nd:YAG laser for tooth whitening, analgesia, and endodontics.
- Frequency-doubled alexandrite laser for selective calculus ablation.

The factors that will determine whether these lasers will gain marketing clearance by the FDA and clinical acceptance are numerous. Apart from safety issues, perceived clinical utility, ease of use, and related expenses for purchase and maintenance will play the key role for their establishment.

B.2 CARIES PROCESS

As shown through the vast literature on the dental caries process, the mechanism of dental caries is in fact quite simple. The primary causative agents of dental caries are two groups of bacteria that inhabit the dental plaque on the tooth surface either separately or together: a) mutans streptococci and b) lactobaccili.[24] Each group contains several species, all of which are cariogenic due to their ability to produce acids as byproducts, when they metabolize dietary fermentable carbohydrates. These acids are predominantly lactic, acetic, and proprionic acids that readily produce hydrogen ions and lower the pH in the plaque rapidly.[25] As a result of the pH drop, acid diffusion into the underlying enamel or dentin and dissolution of the mineral in the subsurface take place. The calcium and phosphate ions of the dental mineral diffuse out of the tooth, leading to a subsurface lesion with altered optical properties, increased porosity that allows absorption of proteins and bacterial byproducts, and decreased mineralization (i.e., incipient caries).[1,26] This process is known as demineralization and continues every time ingestion of fermentable carbohydrates takes place. In enamel, subsurface demineralization typically is first observed clinically as a "white spot" lesion. The body of this subsurface lesion may have lost as much as 50% of its original mineral content while often is covered by an

"apparently intact surface layer".[26] If mineral loss is not halted or reversed by remineralization, (i.e., redeposition of mineral via saliva) frank cavitation may result.

Enamel and dentin tissues contain carbonated hydroxyapatite (CAP) mineral crystals (approximately 85% and 50% by volume respectively), as well as water (8% and 20% respectively) and proteins (amelogenins and enamelins in enamel [2%], and collagen and non-collagenous proteins in dentin [30%]) in between the crystals.[27-29] The CAP mineral contains hydroxyl groups, phosphate, and carbonate (about 3% in enamel and 5% in dentin by weight) in the crystal structure. The carbonate causes structural defects in the crystal lattice that render the mineral more acid-soluble than the pure hydroxyapatite and even more so than fluorapatite, which exhibits the lowest solubility.[30] This property can be exploited by fluoride treatment or laser treatment or a combination of fluoride and laser treatment to change the surface mineral of the tooth permanently to a less soluble form.

B.3 CARIES REVERSAL AND INHIBITION

B.3.1 Role of Saliva

Saliva is of fundamental importance in the inhibition or reversal of the caries process. Saliva provides calcium and phosphate, antibacterial substances, and buffers. It also contains proteins that maintain supersaturation of calcium in the plaque fluid, and proteins that together with lipids form a protective pellicle on the tooth surfaces.[31]

These compounds of saliva neutralize the acidic byproducts of the bacterial metabolism in the dental plaque, raise the pH, and reverse the diffusion gradient for calcium and phosphate. As a result, a remineralization process takes place, during which calcium and phosphate return into the subsurface lesion of the tooth structure and grow new mineral on the surfaces of the crystal remnants produced during the demineralization phase.[1,30] The remineralized crystals now have a veneer of mineral that is much more acid resistant. In addition, saliva clears carbohydrates and acids from the plaque. When normal salivary function is reduced or impaired for various reasons (e.g., diseases, drugs, therapeutic head and neck irradiation because of tumors) all of the above benefits of saliva are reduced or completely eliminated resulting in an extremely high risk for caries development.[32]

B.3.2 Role of Fluoride

The ability of fluoride to prevent and arrest caries has been well established through extensive research. Fluoridation of public water supplies in many communities resulted in caries reductions of 40-70% in the years before the 1970s.[33-36] During the last 20 years, the wide use of fluoride containing products (e.g., dentifrices, mouthwashes, topical gels) has been documented to be a major contributor in the reduction of caries prevalence.[37,38] Fluoride has three principal topical mechanisms of action:

a) Inhibition of bacterial metabolism

When the dental plaque is acidified due to bacterial production of acids, a portion of the fluoride present in the plaque fluid combines with hydrogen ions to form hydrogen

fluoride (HF). While the ionized form of fluoride (F^-) is unable to cross the cell wall and membrane, the uncharged form (HF) can rapidly diffuse into the cariogenic bacteria, effectively drawing more HF from the extracellular environment.[39-41] Inside the bacterial cell, the HF dissociates, acidifying the intracellular environment and releasing fluoride ions that interfere with important enzymatic activities in the bacterium. For example, fluoride inhibits enolase, an enzyme essential for the metabolism of carbohydrates by the bacteria.

b) Inhibition of demineralization

Fluoride present in the aqueous solution surrounding the CAP crystals of the tooth mineral is adsorbed strongly to their surface and protects it from acid dissolution taking place in the subsurface of the tooth. Similarly, fluoride present in the plaque fluid during the time of bacterial acid production travels with the acid into the subsurface of the tooth, where it adsorbs to the crystal surface and protects it against being dissolved.[30,43] Only fluoride that originates from the plaque fluid via topical sources (i.e., drinking water and fluoride products) exhibits a measurable inhibitory effect on demineralization.[30,43] Therefore, fluoride is needed regularly throughout an individuals life to ensure tooth protection against caries.

Fluoride incorporated into the CAP crystals of tooth mineral during tooth development is insufficient to play a significant role in caries protection.[30,44] Sound enamel contains about 10-100 ppm fluoride, depending on the fluoride ingestion during tooth development, except in its outer few micrometers, where the concentration of fluoride may reach a level of 1,000-2,000 ppm.[45] Featherstone et al[42,46], Nelson et al[43], and ten Cate[30] found no measurable reduction in the acid solubility of synthetic CAP (3%

by weight, similar to that of dental enamel) with 1,000 ppm fluoride incorporated. In contrast, as little as 1 ppm of fluoride in the acid solution reduced the dissolution rate of CAP to a rate equivalent to that of hydroxyapatite (HAP)[42], while further increases of fluoride concentrations in the acid solution surrounding the CAP decreased the solubility rate logarithmically.

c) Enhancement of remineralization

Fluoride enhances remineralization by adsorbing to the crystal surface and attracting calcium ions, followed by phosphate ions, which eventually leads to formation of new mineral. As the saliva flows over the plaque, its components neutralize the acid, raise the pH, and stop and reverse demineralization. The saliva is supersaturated with calcium and phosphate, which can drive mineral back into the tooth.[30,47] The partially demineralized crystal surfaces within the subsurface lesion act as "nucleators" and give rise to new surfaces on the crystals. This newly formed veneer excludes carbonate and has a composition somewhere between HAP and FAP. FAP contains about 30,000 ppm F and has a very low solubility in acid. Therefore, the new FAP-like veneer will exhibit a much lower solubility than the original CAP tooth mineral.[42] This means that the remineralized enamel will require rather strong and prolonged subsequent acid challenges to dissolve.

B.3.3 Role of Laser Light

Laser treatment of the tooth surfaces with specific irradiation conditions can enhance remineralization and the effect of fluoride.[48,49] Early studies in the field

indicated that laser irradiation could alter the acid solubility of dental enamel. Yet, these studies were not based on a rational choice of laser parameters, neither were they given much thought in terms of underlying mechanism. Rather, availability of instruments (a ruby laser) and arbitrary selection of usually very high energy densities characterized the experiments.[8,9] Yamamoto showed the potential of an Nd:YAG laser to fuse enamel and decrease its acid solubility but the required irradiation intensities were very high and thus impractical.[10-12]

Since the early 1980s, Nelson, Featherstone, and coworkers strongly advocated the need to establish the scientific basis for the choice of laser types and conditions that could have a clinical use in the prevention or removal of caries lesions. For caries prevention, they advocated lasers whose wavelengths coincided with the major absorption bands of the dental tissues such as CO_2 lasers, as the most efficient and effective ones.[50-53] These researchers' extensive laboratory studies verified their initial hypothesis that: a) there are specific sets of laser irradiation conditions under which the laser light has an optimal interaction with the dental hard tissues; and b) this interaction is mostly thermal in nature and includes efficient conversion of the laser light to heat in the outer few micrometers of tooth surface, leading to thermal decomposition of the enamel crystals to a less soluble form.

Featherstone and Nelson observed that irradiation conditions that produced surface temperature rises in excess of 1000°C increased the acid resistance of dental enamel.[50] At these temperatures, the enamel crystals melted and fused, creating a surface melt zone no deeper then 5μm. Underneath, there was a zone 10-40 μm deep, where the temperature rise was insufficient to cause fusion of the crystals but sufficient for some

compositional changes. As shown by infrared analysis, the carbonate content of the altered crystals was dramatically reduced, and the surface phases present were hydroxyapatite and tetracalcium diphosphate monoxide ($Ca_4(PO_4)_2O$).[51,52]

Other investigators reported comparable effects after heat treatment or laser irradiation of human enamel. For example, Kuroda and Fowler observed a dramatic reduction in the carbonate content of enamel (66%) after irradiation with a continuous wave CO_2 laser. Subsequent x-ray diffraction and infrared analysis indicated that the surface phases were a mixture of minor phases of α-$Ca_3(PO_4)_2$ and $Ca_4(PO_4)_2O$, and modified hydroxyapatite.[54,55] The same investigators described the chemical and morphological changes in dental enamel after heating (in furnace) over a range of temperatures.[54] Between 350-1100°C, there is loss of H_2O and CO_3^{2-}, destruction of proteins, contraction of the lattice, and decomposition of some of the hydroxyapatite to β-$Ca_3(PO_4)_2$, $Ca_4(PO_4)_2O$, and α-$Ca_3(PO_4)_2$ phases. When enamel is heated to temperatures 350-650°C, there is an increase in its permeability.[56,57] In contrast, when enamel is heated above 650°C its permeability decreases.[9] Heat treatment between 350-650°C reduces the solubility of enamel to acid dissolution despite the fact that the permeability is increased.[12,58-60] Conventional heating experiments have shown that 30% of the carbonate is lost at temperatures between 400-600°C and the remaining carbonate is slowly removed at higher temperatures.[61] Thus, it seems that the decomposition of the carbonate groups in the enamel crystals of the surface, which leaves behind a hydroxyapatite-like mineral with less lattice defects, is the most important structural and chemical change to decrease the solubility of enamel. In fact, Featherstone et al. in recent

studies have shown a correlation between carbonate loss due to laser irradiation and a reduced rate of enamel dissolution in acid.[20]

The combination of laser irradiation and fluoride treatment can reduce the solubility of dental enamel to acid dissolution significantly more than either technique independently.[49,62-64] It seems that the laser treatment enhances the incorporation of fluoride ions into the enamel crystals when laser irradiation is followed by application of acidulated phosphate fluoride (APF). Yet, the marked combination effect is lost when the treatments are applied in the reverse order.[63]

Featherstone et al. using an *in vitro* chemical artificial caries model based upon demineralization observed *in vivo*[65], showed that pretreatment of tooth enamel by pulsed CO_2 laser irradiation can inhibit subsequent caries-like progression by up to 85%.[13,66] In that model, at wavelengths of 9.3 and 9.6 μm, as few as 25 pulses of 100 μs duration each, delivered at a repetition rate of 10 Hz, and with fluences between 2.5-5 J/cm^2 were able to produce a preventive effect that is comparable to daily fluoride dentifrice application. Again, in the same model, pulsed CO_2 irradiation followed by subsequent treatment with APF resulted in complete inhibition of caries-like lesion progression.[48] Recently, and for the first time, Featherstone et al. used an intra-oral model to determine whether similar inhibition is observed in the human mouth.[67] In this model, caries-like lesions created *in vitro* in the enamel of extracted human teeth were subjected to irradiation with specific CO_2 laser conditions and subsequently exposed intra-orally, being mounted in the dentures of partial denture wearers. It was shown that pretreatment with 25 pulses (per spot) of 100 μs each, at 9.6 μm, 5 Hz, and fluences 4 J/cm^2 markedly

inhibited subsequent caries-like lesion progression in the human mouth in a fashion comparable to that of a fluoride dentifrice.

A variety of laser conditions can be used to increase the acid resistance of enamel. In general, the effects depend on the characteristics of the laser (e.g., wavelength, pulse duration, number of pulses, etc.). By adjusting these characteristics, both efficiency and pulpal safety requirements can be satisfied (i.e., optimal surface heating with minimal increase of the pulpal temperature that remains below the critical temperature rise for pulp damage of 5.5°C).

Featherstone, Fried, and coworkers have shown that the optimal wavelength for caries inhibition is 9.3-9.6 μm, with pulse durations of 100 μs or less, and incident fluences (energy per surface area, per pulse) of less than 4 J/cm^2.[20,68-70] These conditions cannot be produced by any of the currently available commercial CO_2 lasers, which emit light at just one wavelength of 10.6 μm. For their experiments, the above researchers used different tunable CO_2 lasers designed for laboratory use, able to operate in all four wavelengths (9.3, 9.6, 10.3, 10.6 μm) that correspond to the principal vibrational emission bands of gas phase CO_2 molecules. At present, studies carried out by these investigators are focused on optimizing the irradiation parameters for caries inhibition as well as caries and dental hard tissue ablation. For the past several years, these workers have studied important optical properties of the dental hard tissues as well as thermal effects and thermal modeling, ablation and vaporization at numerous wavelengths.[15,50,69-78] Understanding thoroughly the optical interactions between the laser and the target tissue is of paramount importance to ensure safety and efficiency in any procedure using

lasers. The results from the above and similar studies by other workers will be described briefly in the following section.

B.4 DETERMINANTS OF LASER-HARD TISSUE INTERACTIONS

There are two factors that determine the interaction between laser and tissue:

a) The optical properties of the tissue such as transmission, absorption, scattering and reflection. The optical properties are expressed by the refractive indices of the tissue (n_r, k), the scattering and absorption coefficients (μ_s and μ_α), the reflectance (R), and the scattering anisotropy.[71,72,74,76,79]

b) The irradiation parameters such as wavelength, continuous or pulsed emission, repetition rate, pulse duration, pulse energy, beam size and delivery method, and temporal characteristics of the laser beam.

The ultimate effects of laser irradiation on tissue depend on the distribution of deposited energy inside the tissue. The temperature rise at each point in the exposed tissue is a balance between energy deposited in a specific time and energy conducted away as heat. The temperature rise determines the extent of chemical and morphological changes of the tissue.[15,69,70,78,80]

B.4.1 Dental Hard Tissue Optical Properties

Absorption and scattering coefficients are determined experimentally and are given values with units of reciprocal centimeters (cm^{-1}). Extensive work has been

completed or is in progress to determine the dental enamel and dentin coefficients for several wavelengths of interest.[15,69,70,78,80-85]

In tissues with high absorption for a specific wavelength, the overall energy deposition can be predicted mainly by the absorption coefficient and the tissue reflectance, while the role of the scattering coefficient is negligible. The reflectance (R) of a tissue describes the fraction of incident laser light reflected away from the surface, having no effect on the tissue, and can increase markedly and approach 100% in regions of strong absorption. Tissues that have high absorption coefficients (μ_α>500 cm^{-1}) for a specific laser wavelength, absorb the laser energy at this wavelength within 100 μm of the surface and convert it to heat.[15,69,70,78,79] Light scattering in this case is insignificant, thus, energy transfer deeper into the tissue is primarily due to heat conduction away from this limited surface zone. Whether the deposited heat will remain confined in the surface or will be conducted deeper is determined by the laser wave characteristics (e.g., pulsed or continuous emission, pulse duration, pulse repetition rate, etc.) and the thermal diffusivity and heat capacity of the tissue. Enamel and dentin have marked differences in these characteristics.[69,70,78,86]

On the other hand, light scattering plays a very important role in determining the deposited energy distribution in the tissue, when the tissue has a very low absorption coefficient for a specific laser wavelength. Scattering (the deflection of light in different directions inside the mass of the tissue so that it is lost from the original beam) usually produces no useful biologic effect but it can transfer heat to adjacent tissues (e.g., dental pulp) and cause unwanted thermal damage. In this case, the directional nature or anisotropy of the scattering is also important. Anisotropy is determined experimentally

for each particular tissue and wavelength and it is described by a mathematical function called the "scattering phase function". In biological tissues, the phase function usually contains a single parameter (g), which is the average cosine of the scattering angle. A g value close to one indicates highly forward directed scattering. In practical terms, this implies that transmitted light (i.e., light passing directly through the tissue with no effect on the target tissue) and scattered light can penetrate deep below the surface of the tissue and affect underlying tissues with different optical properties.

Regions and wavelengths with high absorption correspond to specific components of the tissue. For example, dental enamel has a dramatically high absorption coefficient for the 9.6μm wavelength of the CO_2 laser, due to the phosphate ions in the carbonated apatite mineral.[50] The spectral output of the CO_2 laser (9.3, 9.6, 10.3, 10.6 μm) overlaps the strong phosphate absorption bands of dental enamel apatite over the same wavelength range. Also, enamel (and even more so dentin) has a rather high absorption coefficient for the 2.94 μm wavelength of the Er:YAG laser (currently on the US market for ablation of caries in enamel and dentin) due to the free water content of the tissue. Er:YAG laser emission overlaps the broad water absorption band centered at 3 μm. Similarly, enamel and dentin have a rather high absorption coefficient for the 2.79 μm wavelength of the Er:YSGG laser due to the hydroxyl ions (OH^-) of the apatite mineral. Er:YSGG emission overlaps the narrow OH^- apatite absorption band at 2.8 μm.[29,50]

The infrared transmission spectrum of enamel in Figure 1 illustrates the overlap between the absorption bands of the main chemical groups of the enamel, and the position of the laser lines for Er:YSGG, Er:YAG, and CO_2.

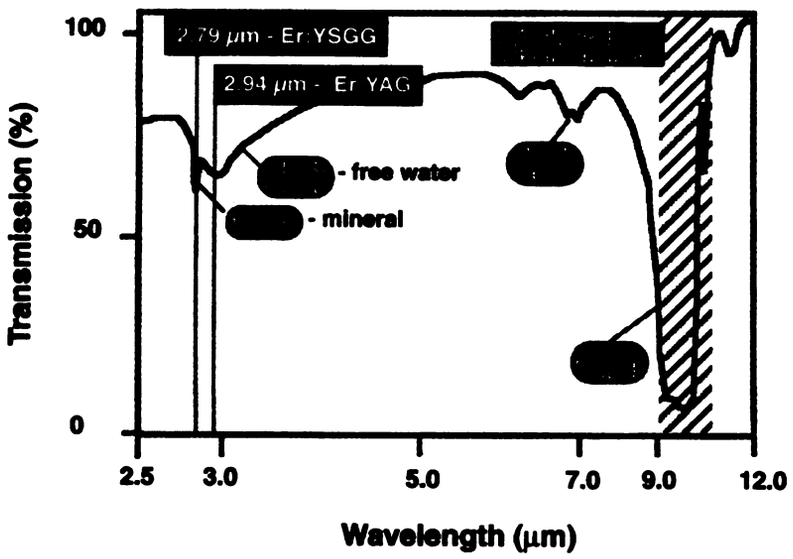

Figure 1. Infrared transmission spectrum of dental enamel showing the absorption bands for water, hydroxyl, carbonate, and phosphate. The positions of the laser lines for Er:YSGG, Er:YAG, and CO_2 lasers is illustrated overlapping with the absorption bands of the enamel.[29,30]

Visible and Near-Infrared

Dental enamel absorbs invisible ultraviolet light in the 240-300 nm region moderately (μ_α~10 cm^{-1}) and visible light in the 400-700 nm region (e.g., the region of argon laser at 488 nm and 514 nm) very weakly (μ_α<1 cm^{-1}). Similarly, enamel exhibits poor absorption (μ_α<1 cm^{-1}) of invisible light in the near-infrared region (e.g., the region of Nd:YAG laser at 1064 nm). The scattering coefficients decrease exponentially in the 240-700 nm region (μ_s=100-40 cm^{-1} at 400-700 nm) and even more so in the near infrared region (μ_s=15 cm^{-1} at 1064 nm).[71,80-82,84,85] These data imply that the light in the visible and near-infrared passes through enamel almost entirely with negligible absorption and moderate scattering, which decreases with increasing wavelength.

In dentin, absorption is low in the visible region but higher than that of enamel (μ_α~4 cm^{-1}). The scattering coefficients for the same wavelength range are higher than

those of enamel (μ_s=250-300 cm^{-1}). Similarly, dentin absorption is low in the near-infrared region (μ_α~4 cm^{-1}), but scattering is significantly higher than that of enamel (μ_s~260 cm^{-1} at 1064 nm).[71,80,84] Seka et al. have shown using Monte Carlo simulations that at the Nd:YAG wavelength, the higher absorption coefficient of dentin coupled with stronger scattering can lead to preferential energy deposition near the dentin-enamel interface.[87] This clinically may translate to some negative consequences such as subsurface vaporization, cracking, and pulpal necrosis.

In both enamel and dentin the scattering of the visible and near-infrared light has high forward directionality. Fried et al. determined the scattering anisotropy (g) for dental enamel and dentin at 543, 632, and 1064 nm as very close to one (g=0.96 for enamel and 0.93 for dentin).[71] Consequently, light in the visible and near IR can penetrate deep into the interior of the tooth.

Mid-Infrared

Both enamel and dentin have high absorption coefficients at mid-infrared wavelengths that coincide with the constituents of the tissue (μ_α=450-8,000 cm^{-1}). In contrast, transmission is low and scattering is non measurable (μ_s~0). Therefore, the absorption coefficient and the tissue reflectance are the main determinants of the energy deposition in this range.[15,78,84]

The absorption coefficient of enamel for the Er:YAG laser light (2.94 μm) has been found to be 770 cm^{-1}, with water being the principal absorber. The rapid and efficient coupling of laser irradiation with the water, causes subsurface superheating, expansion, and explosion, resulting in removal of the tissue.[77,88] The absorption

coefficient of enamel for the Er:YSGG laser light (2.79 μm) has been determined at 450 cm^{-1}, with the hydroxyl ion in the mineral being the principal absorber. The coupling of laser irradiation with the hydroxyl ion results in heating of the mineral as well as of the water. Both avenues (mineral and water) lead to subsurface superheating and effective ablation of the tissue. These properties explain the suitability of these two lasers for dental applications encompassing hard tissue removal.[76,88,89] The reflectance at both 2.79 and 2.94 μm is low, on the order of 5%.[76]

The absorption coefficients of enamel and dentin vary significantly over the spectral range of the CO_2 laser light. Each one of the four CO_2 laser principal wavelengths (9.3, 9.6, 10.3, 10.6 μm) can couple with the phosphate ion of the carbonated apatite (CAP) dental mineral. The phosphate ion in CAP has three vibrational modes at 9.2, 9.6, and 10.4 cm^{-1}, which are responsible for the strong absorption band in the emission range of the CO_2 laser and the observed differences in the absorption coefficients for each CO_2 wavelength. The very efficient and rapid coupling of CO_2 laser irradiation with the phosphate changes the chemical composition of the dental mineral decreasing the solubility to acid dissolution as previously described. This property explains the potential of specific wavelengths of the CO_2 laser for caries preventive treatments. Under certain irradiation conditions, the laser induced thermal decomposition of the tissue components and the thermal expansion of the tissue water may lead to rapid release of subsurface gases, such as water vapor and CO_2 into the pores of the tissue. The highly increased pressure in the pores may cause explosive removal of material.[89] This property of the CO_2 laser can be exploited to preferentially remove carious tissue, which contains water and protein at a higher ratio than normal tissue. Combined these

properties of the CO_2 laser may allow for very conservative preparations with smooth surfaces around the periphery, which have enhanced resistance to caries. The absorption coefficients of enamel for the 9.3, 9.6, 10.3, and 10.6 μm wavelengths of CO_2 laser light have been determined to be approximately 5500 cm^{-1}, 8000 cm^{-1}, 1125 cm^{-1} and 825 cm^{-1} respectively.[15,78] The absorption at 9.3 and 9.6 μm is an order of magnitude higher than the absorption at 10.6μm, which is the only CO_2 wavelength available commercially at present. This implies that laser light of 9.3 and especially 9.6 μm is much more suitable than that of 10.6 μm for applications that require efficient and short heating of the mineral, such as caries preventive treatments. The reflectance at CO_2 wavelengths can be very high (38% at 9.3 μm, 49% at 9.6 μm, 16% at 10.3 μm, and 13% at 10.6 μm) therefore, it has to be seriously taken into account in energy calculations and in safety issues during laser operation.[74,89,90]

B.4.2 Irradiation Parameters

For a given target tissue, judicious selection of laser type and manipulation of related spatial and temporal variables of the laser beam, enables the operator to have precise control over the laser-tissue interaction to achieve the desired optimal effects.

Wavelength

Wavelength is a fundamental characteristic of the laser light (and of any type of electromagnetic energy). Each type of laser emits a characteristic wavelength or a, usually, small range of wavelengths that depend on the type of material that emits the

laser light. Some lasers can be tuned in one or another wavelength by adjusting the laser's optical system. The wavelength dictates to a great extent the properties of laser light and therefore the type of interaction with the target tissue. When the desired interaction is the efficient conversion of laser energy to heat for purposes such as tissue surface modification or vaporization, the laser light must be absorbed strongly by some component or components of the tissue. As has already been described, dental enamel is predominantly carbonated hydroxyapatite mineral, which most strongly absorbs laser light in the 9.3-10.6 μm range (especially the 9.6 μm wavelength). This is why the CO_2 laser is expected to be most useful amongst all other available laser types for modification of enamel to provide caries resistance.[13,14]

The wavelength(s) produced by each laser type dictate the availability of choices in terms of delivery system in the clinical set-up. Dental lasers use one of three delivery systems. One is a flexible hollow wave-guide, which delivers the laser beam to the target in a non-contact fashion. The second is an articulated arm system with mirrors at the junctions. These two delivery systems deprive the operator of tactile sensation and require heightened attention during operation to ensure use at the "focal spot" of the laser, where the emitted energy is greatest. The third delivery system is a glass fiberoptic, pliable cable, which can be used in contact or non contact fashion depending on the intended result on the tissue (e.g. cutting versus coagulation). Contact use of a laser offers the advantages of tactile sensation, higher precision, and easy access to otherwise difficult to reach areas of tissue. The "focal spot" in this case is at or near the tip of the fiber, which makes handling less attention intensive for the operator. Most invisible dental lasers are equipped with a separate aiming beam, coaxial to the wave-

guide or fiber, in order to aid the operator in the localization of the focal spot of the laser. Lasers with shorter emission lengths such as argon, diode, and Nd:YAG can be designed easily with small flexible glass fibers. As the wavelength increases the fiber technology is challenged because large waves do not fit into the molecules of the conducting glass fiber easily. The CO_2 wavelengths are too long for glass and have to be conducted in a hollow tube, either a wave-guide or articulated arm.

Energy and Fluence

The primary beneficial effect of laser energy on biologic tissues is photothermal in nature, due to absorption of the laser light by the tissue of interest. There are also other effects that lasers have on selected tissues. Amongst them are photochemical effects (e.g., stimulation of chemical reactions and breaking of chemical bonds) and photoacoustic effects (e.g., production of a shock wave within a hard tissue and creation of an ablated crater). For the purpose of dental caries prevention, the photo thermal effects of laser energy are the ones of main interest. In order to make the mineral of dental enamel resistant to acid dissolution, the aim of laser treatment is to raise the temperature of the enamel at and near the surface enough to beneficially alter the mineral, but without causing excessive ablation or undesirable peripheral damage. Experimental work with enamel and synthetic carbonated apatite has indicated that surface temperatures in the range of 400-1200°C are needed to achieve structural modification of the mineral and increased acid resistance.[50-52,58,91] Melting of enamel mineral occurs in the range of 850-1200°C and vaporization occurs above 1600°C.[92,93] Fried et al. have shown, via thermal radiometry measurements on enamel during laser irradiation, that

fluence has a major effect on surface temperature profiles.[16,17] Fluence or energy density is an expression of energy in terms of total energy delivered per unit area of the irradiated target, and it is measured in Joules/cm^2. In many instances, fluence is one of the most important parameters for laser therapy. Based on the above results, Featherstone et al. used a range of fluences and wavelengths to study the laser-induced inhibition of caries progression in artificial caries models. Within that range, and with the rest of the irradiation parameters being fixed at a pulse width of 100 μs, a repetition rate of 10 Hz, and a total number of 25 pulses per spot, optimum inhibition of caries was achieved at 9.3 and 9.6 μm and incident fluences of 2-6 J/cm^2 per pulse.[13]

Emission Mode and Pulse Temporal Characteristics

Laser energy is emitted in one of three basic modes: a) continuous-wave mode, b) gated-pulse mode, and c) free-running pulsed mode. In *continuous-wave mode* the laser beam is emitted continuously at a steady energy level for as long as the device is activated. In *gated-pulse mode*, periodic alternations of the emitted laser energy are introduced by the opening and closing of a mechanical shutter in front of the beam path of a continuous-wave emission. The on and off intervals of this laser are usually in the order of a few milliseconds. In *free-running pulsed mode*, large peak energies of laser light are emitted for an extremely short span (usually microseconds), separated by a relatively long interval in which the laser is off. The timing of this emission is generally computer controlled. The mode of operation allowable to a laser type depends on both the physics of the laser and the design of the laser excitation mechanism. In practice, some lasers operate only in pulsed mode because they cannot sustain steady laser

emission. Others operate only in continuous wave mode because it takes time to establish the right conditions for laser oscillation. Some lasers can operate in either mode depending on operating conditions (e.g., CO_2 laser).

When the purpose of laser treatment is dental enamel modification for caries resistance, the laser of choice is a free-running pulsed laser. Pulsed lasers provide a way of increasing the peak power density (where power density is the amount of power [energy per time] that is concentrated into a spot), while keeping the fluence constant. This practically means that with proper selection of the **pulse duration,** the laser energy deposition can be confined to the tissue surface, inducing the desired thermal changes on the outer few microns without affecting the underlying dentin or dental pulp. The optimal pulse duration should be in the range of the thermal relaxation time for axial heat conduction (t_r) of the deposited energy in the tissue surface. The thermal relaxation time is the length of time required for the temperature of the surface layer of thickness $1/\mu_\alpha$ to drop to approximately half the initial temperature. If the pulse duration is longer than t_r, the laser energy is conducted away from the enamel surface into the interior of the tooth during the laser pulse. This results in inefficient surface heating and increased risk for pulpal damage due to excessive heat accumulation in the pulp. On the other hand, if the pulse duration is much shorter than t_r, the deposited power density (energy per time per area) may be too high, resulting in ablation of tissue instead of the desired heating and fusion. Fried, Zuerlein, et al. have calculated the thermal relaxation time for dental enamel to be 1 μs for the 9.6 μm wavelength and about 90 μs for the 10.6 μm wavelength.[15-18]

Most CO_2 lasers used in earlier studies emitted light at 10.6 μm in continuous wave mode with typical tissue interaction times of 50 ms to 2 s. These interaction times were much longer than the thermal relaxation time of enamel. In continuous emission the deposited energy has a linear relationship directly proportional to the total time of exposure.[19] Therefore, such treatments result in greater heat transfer to surrounding tissues and pulpal damage from excessive heat accumulation in the pulp.

The **repetition rate** (i.e., the number of pulses per second) needs to be slow enough to permit cooling between pulses but fast enough to keep the overall irradiation time short and thus, clinically useful. Early studies by Nelson et al. showed significant enamel caries inhibition in artificial caries-like laboratory models by use of pulsed laser irradiation at 9.6 μm.[50-53] However, because of limitations imposed by the CO_2 laser system available, low fluences per pulse were used (0.12 J/cm^2), with low repetition rates (0.6 Hz), and very short pulse durations (100-200 ns), necessitating a high number of pulses (200-400) for measurable effects. Under those conditions, irradiation of a single spot took 10-20 minutes, which would be impractical in terms of clinical reality. Later experiments by Featherstone et al. with CO_2 lasers capable of higher repetition rates and fluences per pulse showed much higher caries inhibition with only 25 pulses per spot, incident fluences as low as 2.5 J/cm^2 per pulse, a pulse duration of 100 μs, and a repetition rate of 10 Hz.[13] These treatments utilized a 2.5 s irradiation time per spot, which would be more practical for clinical use.

The **number of pulses** per spot needs to be high enough to provide the desired surface and subsurface effect, but low enough to keep the total energy deposited to the minimum required, and thus avoid overheating the pulp. Kantorowitz et al. explored the

effect of the number of pulses on the caries preventive potential of CO_2 laser irradiation by use of an artificial caries-like model.[66] Irradiation of enamel surfaces at 9.6 and 10.6 μm, with incident fluences of 5 and 12 J/cm^2 per pulse respectively, a pulse duration of 100 μs, and a repetition rate of 10 Hz, indicated optimal caries inhibition achievable with less than 25 pulses per spot. This study also indicated that there is a point at which further increase in the number of pulses does not increase caries inhibition further. Earlier surface radiometry studies by Fried et al. indicated that at 9.3-10.6 μm, with a pulse duration of 100 μs, and a repetition rate of 10 Hz, a minimum of 10 pulses per spot would be necessary to provide ideal temperature rise at the enamel surface for caries prevention.[16] Featherstone et al. examined the effect of the number of pulses per spot on the acid-solubility of enamel surfaces by use of initial surface dissolution experiments.[20] Irradiation at 9.3 μm, with an incident fluence of 4 J/cm^2 per pulse, a pulse duration of 100 μs, and a repetition rate of 10 Hz, achieved no further measurable decrease in surface dissolution rates beyond a total number of 5 pulses per spot.

Overheating the pulp during laser treatment is the single most important concern of laser irradiation. Studies have shown that the rate of deposition of the laser energy, the distance from the laser spot to the pulp, and the rate of energy loss from the tooth, all determine the extent of pulpal heating.[94-100]

Correlation of pulp temperature stress measurements in Rhesus monkeys with histologic changes revealed that a rise of 5.5°C in pulpal temperature results in pulp necrosis 15% of the time.[101] Since that 1965 study, this 5.5°C temperature rise has often been quoted as a temperature threshold not to be exceeded during dental treatments, whether conventional mechanical means or lasers are used.

Miserendino et al. observed that continuous wave CO_2 laser exposures of the buccal surfaces of extracted molar human teeth produced an increase in pulpal temperature proportional to the level of exposure.[19] Laser exposures of relatively high energy level (30-250 J) resulted in intrapulpal temperature rises of 5.5-32°C, which would be expected to result in irreversible injury to vital structures. Laser exposures below 10 J produced temperature rises less than 5.5°C, which might fall within the range of pulpal tolerance. In those experiments, the power settings (energy per time) ranged from 2-10 W, and the duration of exposure from 0.5-25 s for a 1.0 mm spot size. Measurable thermal changes occurred at power levels above 2 W and exposures longer than 0.5 s. The onset of temperature rise in the pulp chamber occurred within 10-20 s after termination of irradiation and reached maximal levels within 60 s. At lower power settings (2-6 W), return to initial pulpal temperatures required up to 2 min for cooling. At higher power settings (8-10 W) and long durations (10-25 s) cooling required 3-5 min. In a subsequent study, Miserendino et al. evaluated common cooling mechanisms and laser pulsing as methods to facilitate dissipation of laser-generated surface heat and avoid thermal transfer to the pulp.[102] Application of air/water cooling for 5 s after continuous wave CO_2 laser exposures of 5 s at energy levels of 10-50 J (power 2-8 W) reduced the potential for thermal damage to the pulp to levels comparable to that occurring during use of high speed dental rotary instruments. Laser pulsing failed to show any advantage over continuous wave irradiation in terms of decreasing the rise in pulpal temperature. Yet, those experiments employed very long pulse durations (5-25 ms) and high energy levels per pulse (10-50 J), which are inappropriate for caries preventive treatments according to the current knowledge about the optical properties of dental enamel.

Fried et al., with thermocouple measurements, demonstrated that caries preventive eatments requiring multiple pulse irradiation can be applied with minimal risk to pulpal ssues by using the highly absorbed CO_2 wavelengths of 9.3 and 9.6 μm.[75] The most mmonly used 10.6 μm wavelength exhibits a higher risk of cumulative heating that ay endanger the pulp. In fact, for the same pulse duration (100 μs), repetition rate (10), and spot size (2 mm^2), irradiation at 9.6 μm with an incident energy of 50 mJ per se resulted in a 9-fold decrease in the cumulative temperature rise 2 mm from the diated tooth surface as compared to irradiation at 10.6 μm with an incident energy of mJ per pulse.[75] Thermocouple measurements have been useful in setting an upper it on the temperature rise in the pulp chamber after a laser treatment. Their usefulness omewhat shadowed by the lack of the heat losses and sinks present in vivo (i.e., blood w, blood perfusion, conduction through bone, water and air cooling). Those factors uld reduce the expected temperature rise. Therefore, the thermocouple studies offer a servative guideline for heat flow estimates, yet, their value is in no way diminished.

C. EXPERIMENTAL DESIGN AND METHODS

C.1 EXPERIMENTAL OUTLINE

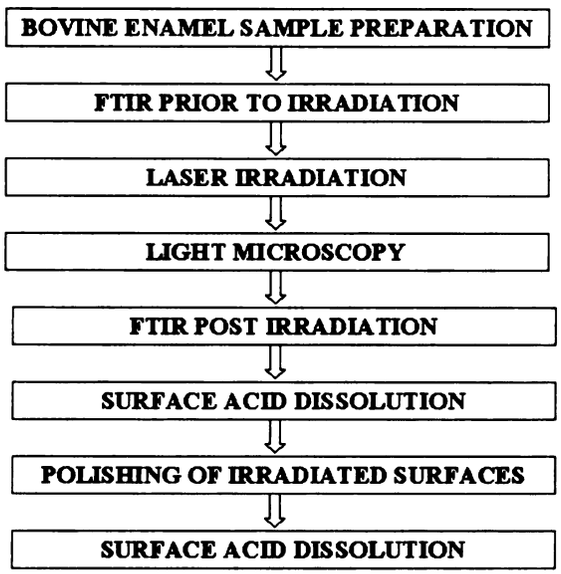

Table 1. Overview of experimental procedures

1) Bovine enamel blocks were serially polished to achieve uniformly smooth surfaces and serve as experimental samples.

2) All samples were analyzed before irradiation by Fourier transform infrared spectroscopy (FTIR) in specular reflectance mode to determine the carbonate content of the surface mineral.

3) Subsequently, the samples were treated with CO_2 laser irradiation by specific laser conditions. At least three samples were irradiated for each set of conditions, so that a mean could be deduced for each irradiation scenario.

4) The irradiated samples were inspected under a light microscope for surface changes, and the images were stored in a computer with image processing software.

5) All irradiated surfaces were analyzed by FTIR to determine changes in the carbonate content of the surface mineral as a result of irradiation.

6) The irradiated samples were subjected to surface acid-dissolution for calcium and phosphate dissolution-rate measurements.

7) The business surfaces of selected experimental samples were serially polished to even smoothness, as previously described, and subjected anew to acid dissolution-rate measurements to serve as controls. The initial intention was to obtain "irradiated" and "polished" values for each individual sample. Time constrictions limited the gathering of "polished" dissolution data to only certain samples. In addition, acid dissolution data was gathered from a number of enamel blocks that were not irradiated or otherwise treated, to serve also as controls.

8) The dissolution data was statistically analyzed to infer significance of differences as a result of different irradiation conditions.

C.2 SAMPLE PREPARATION

Bovine enamel blocks 5x5x2 mm were prepared, and serially polished with a Buehler Ecomet II Polisher/Grinder to achieve uniformly smooth surfaces. The smooth rotating wheels of the apparatus were outfitted with sanding papers of different grits, and cloths designed to be used with polishing agents. A suspension made by Buehler was used as a polishing agent. This suspension contains diamond particles in a water-soluble

base, and may come in different grits (e.g., 6-1 μ polish). When transitioning from one set of polishing conditions to the next finer grit, the samples were washed with Ivory bar soap and double-deionized water (DDW), and then sonicated in DDW for 1 minute. Ivory soap is an ideal cleanser because it is a pure soap with no additives and effectively removes particles from the sample surface. Removal of particles by washing and sonicating at the end of each polishing step is important because it prevents contamination of the subsequent polishing cloths. A dust particle can be as large as 25 μm and can scratch a sample so badly that polishing has to be repeated from the initial grit with the risk of destroying the samples.

Polishing of the business surface of the enamel blocks started with a 600-mesh Silicon Carbide paper in the Ecomet Polisher for 2 minutes. Each block was hand-held firmly on the polishing paper and occasionally rotated slowly on the polisher to ensure uniform polishing. DDW was sprayed on the polisher to prevent the surface from drying. At the end of this polishing step, the block was washed with soap and a soft toothbrush, sonicated for 1 minute, washed again, and checked under the microscope for scratches. If the large scratches had been removed, the polishing proceeded to the next step. The Silicon Carbide paper was replaced by a 6 μm Buehler paper, sprayed with 6 μm Buehler diamond suspension. Each block was polished as before for 4 minutes, all steps being repeated as described. The serial polishing continued with a 3 μm Buehler paper and 3 μm Buehler diamond suspension for 3 minutes, and 1 μm Buehler paper and 1 μm Buehler diamond suspension for 2 minutes. At the end, the polished surfaces were checked again under the microscope for smoothness. If scratches were detected, the polishing was repeated from start to finish in the same order. During subsequent

handling of the blocks, powder free gloves or tweezers were used to prevent skin oils from contacting the polished surfaces and potentially interfering with the experimental processes. Selected enamel blocks were stored individually in labeled polystyrene test tubes with caps, under thymol-humid conditions to maintain hydration and limit microbial growth.

C.3 REFLECTANCE FTIR SPECTROSCOPY

Featherstone et al. have found that FTIR spectroscopy in specular reflectance mode is well suited for resolving chemical changes on the surface of enamel.[20,68] Reflectance FTIR spectroscopy can be used to determine changes in absorption bands of the mineral related to specific chemical species, and to subsequently determine the change in carbonate content. The carbonate content of the mineral can be estimated from the spectrum in the region of the carbonate absorption bands at about 1400 cm^{-1} (approximately 7 μm). Integration of the area under the curve for these bands permits calculation of the relative carbonate loss that may result from laser irradiation, based on normalization to the carbonate bands of the non-irradiated samples. The principal advantage of this spectroscopic technique is that only one factor influences the tissue reflectance, namely a surface layer of a thickness on the order of the wavelength of the light. Therefore, the technique can probe surface changes localized to the outer 10 μm of tissue. Featherstone et al. have found that there is a direct correlation between the changes in the carbonate bands of the spectrum for enamel and the transformation of the carbonated hydroxyapatite mineral to the purer phase hydroxyapatite, which is more acid

resistant.[20] In fact, it seems that the fluence that caused complete elimination of the carbonate bands coincided with the fluence that gave the highest inhibition of both surface and subsurface acid dissolution. For this reason, the FTIR seems to be a very useful nondestructive probe to provide a rapid feedback in relation to optimal laser parameters for caries inhibition.

For the purposes of the present study, an infrared microscope (XAD Plus, Laser Precision Analytical, Irvine, Ca) with a 150x150 μm aperture attached to an FTIR spectrometer (RFX-30, Laser Precision Analytical) was used to obtain infrared spectra of the experimental bovine enamel samples just prior to and right after laser irradiation.

C.4 OPTICAL MICROSCOPY

To obtain images of the irradiated enamel surfaces with magnifications up to 500x, a CCD imaging camera (Olympus U-TV0.5x, Japan) attached to an optical microscope (Olympus BX50, Japan) was used. These images were transferred to a computer with image processing software (Bioquant TCW, R & M Biometrics) for further evaluation of surface changes resulting from laser treatment.

C.5 SURFACE ACID DISSOLUTION

In dental caries, the rate of dissolution of enamel crystals by plaque acids is one very important factor that determines the progression of a carious lesion.[103] In the oral environment, the acid produced during plaque metabolism regularly challenges the outer

few micrometers of dental enamel and causes reformation of the crystal surfaces as the pH alternates between drops and rises. The degree of crystalline disorder (such as the one caused by carbonate substitutions) directly influences the reactivity of enamel to acid. Carbonate inclusion in apatite significantly changes its properties and increases its solubility.[28,29,104,105]

Numerous investigators have studied the kinetics and thermodynamics of enamel dissolution and described it through mathematical formulas. Human, bovine, or synthetic apatite models such as hydroxyapatite or carbonated hydroxyapatite have been used for this purpose. Gray made one of the first quantitative descriptions of bulk enamel dissolution.[106] This first semi-empirical equation was successful enough to soon lead other workers to more detailed mathematical models.[107,108] The development of rotating disc experiments eliminated several of the problems of those initial experiments by establishing well-defined hydrodynamic conditions, and advanced further the understanding of enamel dissolution.[109-113] The enamel dissolution described by these works concerns only surface dissolution, which bears little resemblance to the development of a carious lesion. In the natural lesion, the enamel is dissolved from the sub-surface, while the decalcified region is covered with a relatively sound surface layer. To mimic subsurface decalcification of enamel and production of a white spot without incurring surface damage, *in vitro* artificial caries systems were developed.[114-116] Subsequently, the kinetics of incipient caries-like lesion formation was studied.[117-119] From comparison of the initial rate equations for surface and sub-surface dissolution it seems that both processes are the same at the level of enamel crystallites, the difference lying in the extra diffusion steps in the case of sub-surface dissolution.

In the present study, a specially constructed dissolution apparatus was used to measure the acid reactivity of bovine enamel in acetate buffer and the influence of CO_2 laser irradiation on the initial dissolution rate.[46,120-122] The bovine enamel samples were mounted on high-density-polyethylene disks (HDPE) with a drop of clear 5-minute curing epoxy resin (Devcon, ITW Performance Polymers, FL). All exposed surfaces of the enamel block except for the business surface were coated with an impermeable acid-resistant nail polish/varnish. This nail enamel is manufactured by Revlon Consumer Products Corporation, crème #3994, "Pinkish" #54, and its use for the above type of experiments was validated through pilot studies in the past. The mounted samples were placed (one at a time) in the reactor vessel of the dissolution apparatus containing a buffer solution of 0.1 M acetic acid at a pH of 4.5, and a temperature of 37°C (200 mL per sample). Constant stirring at 300 rpm was routinely used and a N_2 gas line provided steady but light stream of gas bubbling through the liquid, while 2.5 mL aliquots of the solution were collected for 20 minutes at 2-minute intervals after immersion. An aliquot was always taken at time zero just prior to sample placement in the apparatus. The aliquots were analyzed for calcium by atomic absorption and phosphate by UV spectrophotometry to determine the dissolution rate (see below for details).

C.5.1 Preparation of Dissolution Solutions

A) Bulk Solution

Bulk solution was prepared by mixing 58 mL glacial acetic acid (Glacial VWR scientific, Lot 8285) with 700 mL DDW in a clean, dry 1000 mL beaker under constant

stirring. Measurements of pH were made with a Tim 900 pH meter (Radiometer, Copenhagen) that was previously calibrated to pH values of 4.00 and 7.00 using pH standards from Fisher Scientific. The pH was adjusted to 4.00 using 10% NaOH, or 1% HCl. Subsequently, the solution was brought to 1 L volume within a 1 L volumetric flask by addition of DDW, mixed thoroughly, and stored in a clean, plastic (HDPE) bottle at room temperature until needed.

B) Buffer Solution

Buffer solution was prepared by mixing 700 mL of DDW with two volumetric 50 mL aliquots of bulk solution (total 100 mL). The pH was checked and adjusted to 4.50 (as above). Also, 7.456 g KCl were added to maintain a constant background ionic strength and facilitate the subsequent calcium analysis by atomic absorption. The solution was brought to 1L volume in a volumetric flask adding DDW, and was stored in appropriately labeled plastic bottles in an incubator at 37° C.

C.5.2 Calcium Analysis by Atomic Absorption Spectrophotometry

A Perkin Elmer 3110 Atomic Absorption (AA) Spectrophotometer with a nitrous oxide flame was used to determine the calcium concentration in the dissolution aliquots of each sample. The instrument was calibrated using calcium standard solutions of 0.5, 1, 2, and 5 ppm, which were prepared by diluting the 1000 ppm calcium standard solution (Fisher Calcium Reference Solution) with 1000 ppm KCl solution. The presence of potassium in all the measured solutions is necessary to suppress ionization of calcium and

ensure that the calcium remains in its atomic state in the hot nitrous oxide flame. (The specific wavelength absorption of the instrument measures quantitatively the presence of elemental calcium.) The values recorded by the instrument were converted, using the standard calibration curve, to parts per million (ppm) calcium.

C.5.3 Phosphate Analysis by UV Spectrophotometry

A Milton Roy Genesys 5 UV Spectrophotometer was used at a wavelength of 820 nm to determine the phosphate concentration in the dissolution samples. The instrument was calibrated using standard solutions of 0, 1, and 2 ppm (6 standards per assay). All standards and samples were prepared, prior to analysis, with Reagent C dispensed with an Eppendorf repeating pipetter to special cuvettes, and stored in an incubator at 37°C for at least 2 hours before spectrometry. Reagent C contains ammonium molybdate, which together with phosphate make a colored complex that absorbs light at 820 nm wavelength.[123] Reagent C has to be prepared fresh for each assay and be kept refrigerated out of direct light till ready to use. For 20 mL of Reagent C, 8 mL of DDW need to be well mixed with 4 mL of 6 N Sulfuric Acid (H_2SO_4), 4 mL of 10% Ascorbic Acid, and 4 mL of 2.5% Ammonium Molybdate. For that purpose, a disposable 50 mL conical centrifuge tube thoroughly stirred in the vortex mixer can be used, or a glass beaker stirred with a magnetic stir bar on a stir plate. One mL of Reagent C was pipetted into all assay cuvettes. In addition, the cuvettes carrying the samples contained 0.5 mL of the respective dissolution aliquot and 0.5 mL of DDW, while the cuvettes carrying the standards contained 0.5 mL buffer solution. Also, the 0 ppm standard contained 0.5 mL

DDW; the 1 ppm standard contained 0.25 mL DDW and 0.25 mL phosphate (P) standard 2 ppm; the 2 ppm standard contained 0.5mL phosphate (P) standard 2 ppm. Reagent C is the catalyst that begins the reaction, so it is added to the assay just prior to incubation to ensure that all samples are exposed to the catalyst the same amount of time. The concentration of phosphate in each sample was calculated according to the following: Sample Concentration (ppm)=(recorded value of sample-average value of 0 ppm)/(average value of 1 ppm-average value of 0 ppm)

All dissolution data was plotted as either calcium or phosphate concentrations versus time, and the slopes were calculated by linear regression to determine the initial dissolution rates of Ca and P lost into the acid solution during each dissolution run (IGOR Pro 3.1 software, Wave Metrics, Inc.).

C.6 CARBON DIOXIDE LASER TREATMENT

A sealed TEA laser designed and manufactured by Argus Photonics group (Jupiter, FL) was used to irradiate the bovine enamel samples. TEA lasers are compact sources of intense pulses lasting 40 ns to 10 μs. Their ability to generate such short pulses, in contrast to the conventional CO_2 lasers, is due to increased gas pressure to around one atmosphere, and excitation by a pulsed electric discharge transverse to the laser axis. Because of that mechanism of operation, such lasers are called "transversely excited atmospheric-pressure" or "TEA" lasers, although the internal gas pressure is not always one atmosphere. Their power levels, size, and price can cover a wide range.[124]

The laser was tuned to wavelengths of either 9.6 μm or 10.6 μm. The irradiation wavelengths were chosen based on absorption coefficients. In particular, the 9.6 μm wavelength was selected because it is the most highly absorbed by dental enamel and thus, it was expected to be the most efficient for surface modification with minimal energy expenditure. The 10.6 μm wavelength was selected because it is the least absorbed by enamel among the CO_2 wavelengths and thus, it was expected to illustrate more dramatically the difference in energy efficiency compared to 9.6 μm. The TEA pulsed system tested was inherently capable of 2 μs (1-4 μs) pulse durations. This is very close to the thermal relaxation time of enamel for the 9.6 μm wavelength (1 μs).[15-19] Thus, 2 μs pulse durations at 9.6 μm were expected to be able to use much less energy than the longer pulses (100 μs) to produce similar effects. For this reason, incident fluences between 0.1-1 J/cm^2 per pulse were used. (When 100 μs pulses were used, maximum inhibition of dissolution was achieved with 3-5 J/cm^2 per pulse.[13,20]) At 10.6 μm, where the thermal relaxation time of enamel is longer (~90 μs), the shorter pulses were not expected to offer any obvious advantage over longer pulses in terms of energy requirements for similar effects. Here, the selected incident fluences ranged from 0.4-4 J/cm^2 per pulse.[13] A total number of 5 pulses were delivered per spot. A previous study has shown that at 9.3 μm (where the absorption coefficient and reflectance of enamel approximates closely those at 9.6 μm), when the fluence was fixed at 4 J/cm^2, more than 5 pulses of 100 μs pulse duration had minimal further beneficial effect on surface modification.[20] A repetition rate of 1 Hz was specifically chosen for two reasons: a) the laser system in use was not capable of more than 5 Hz, and b) it was desired to avoid heat accumulation effects and isolate primarily the fluence effect as related to this particular

(short) pulse duration.[75] The laser energy was measured and calibrated by laser calorimeters, and the laser spot size was measured by directly imaging the beam with a pyroelectric laser beam profilometer as well as by scanning a razor blade across the beam. The beam profile was single mode, and fluences were defined by using a Gaussian beam with a $1/e^2$ beam diameter. The laser pulse temporal profiles were measured with an Ultrafast pyroelectric detector and a room temperature HgCdTe detector. The laser beam was focused to a 600-1000 μm spot size that was scanned every 200 μm across the sample to treat the entire surface in a uniform way. For the scanning, a Newport MM-2000 motion control system was used, interfaced to Labview 4.0 software from National Instruments.

C.7. SAMPLE SIZE

Thirty two samples were irradiated at 9.6 μm and at incident fluence levels of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, and 1 J/cm^2 per pulse (energies 0.71-7.1 J). At least three samples were included in each fluence group to obtain a useful mean value. All samples were analyzed with FTIR spectroscopy before any treatment and immediately after irradiation, and were exposed to acid surface dissolution after irradiation. From these samples, 10 were polished and initial dissolution rates were measured for a second time to serve as non-irradiated controls.

Twenty one samples were irradiated at 10.6 μm and at incident fluence levels of 0.4, 0.8, 1.2, 1.6, 2, 3, and 4 J/cm^2 per pulse (energies 1.4-14 J). At least three samples were included in each group except for the 1.2 J/cm^2 group that included only one sample

due to complete mechanical failure of the laser system and interruption of irradiation. All samples were also analyzed with FTIR spectroscopy before and immediately after irradiation and exposed to acid surface dissolution after irradiation. From these samples, 4 were polished and initial dissolution rates were measured for a second time to serve as non-irradiated controls. In addition, 8 more samples that had no previous laser or other treatment and were from the same batch as the above 21 enamel blocks were also measured to obtain control values for dissolution rate measurements.

C.8 STATISTICAL TREATMENT OF DATA

Calcium and phosphate dissolution profiles were compared by use of the Multiple Analysis of Variance (MANOVA) test with the Honest Significant Difference for Unequal Group Sizes (unequal NHSD) post hoc test for examining differences between the treatment groups and controls. The tests were done with the assistance of "Statistica for Windows" (V.5.5; StatSoft, Tulsa, OK) statistical software. The reduction in dissolution rates of the experimental groups (irradiated) compared to the control groups (non-irradiated) gives a measure of the efficacy of the laser treatment.

D. RESULTS

D.1 FTIR MEASUREMENTS

Relative carbonate loss as a result of irradiation was calculated by integration of the area under the curve for the carbonate bands and normalization to the carbonate band of the non-irradiated samples (Fig. 2). An almost complete loss of carbonate was evident at incident fluences 0.5-1 J/cm^2, 9.6 μm and 3-4 J/cm^2, 10.6 μm (Fig. 3). This finding correlates well with the dissolution rate results (see section D.3), which indicate an increased resistance to acid dissolution from 0.5 to 1 J/cm^2 for the 9.6 μm wavelength, and from 3 to 4 J/cm^2 for the 10.6 μm wavelength.

D.2 OPTICAL MICROSCOPY IMAGES

All irradiated bovine enamel surfaces were inspected under the optical microscope with magnifications up to 500x and the respective images were stored via an image processing software (Bioquant TCW) to a computer connected to the microscope.

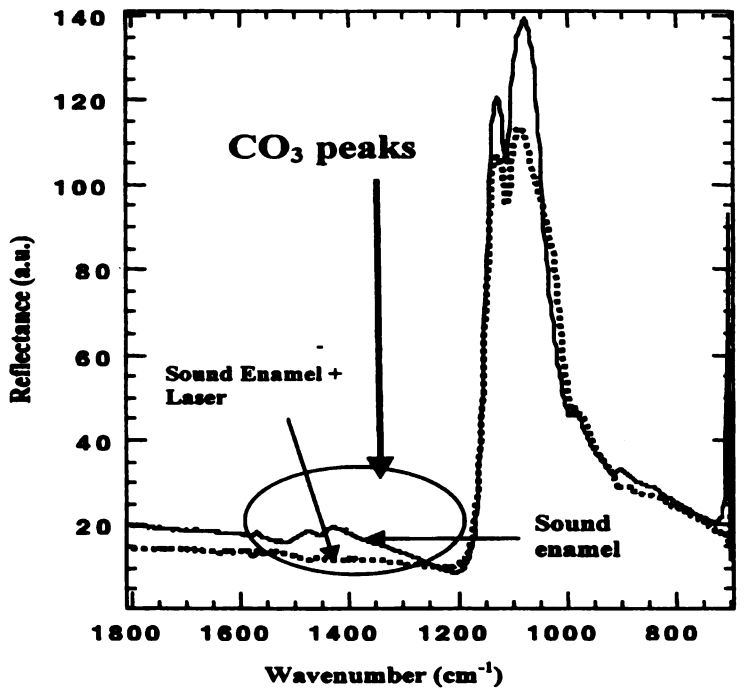

Figure 2. Specular reflectance FTIR spectra of a bovine enamel sample before and after irradiation with 9.6 μm and 1 J/cm². Details of carbonate bands are indicated by arrows. Complete loss of carbonate is evident after irradiation.

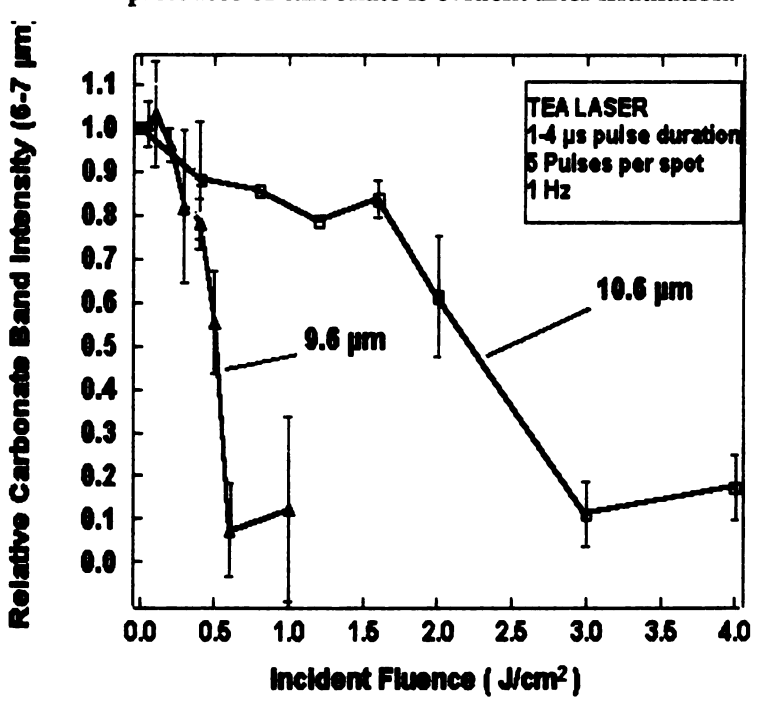

Figure 3. The carbonate loss relative to non-irradiated control samples for bovine enamel specimens is plotted vs. incident fluence after 9.6 μm (triangular symbols) and 10.6 μm (square symbols) CO_2 laser irradiation. Error bars are standard deviations.

After irradiation at 9.6 μm, modification of irradiated surfaces was detectable for fluences 1-0.3 J/cm^2 (Figs. 4 and 5). For example, at fluence 1 J/cm^2 all samples exhibited an almost complete melting of the irradiated surface that in high magnifications appeared as wavelike structures. At fluence 0.6 J/cm^2 the irradiation effect on the surface morphology was still very prominent. Although the surface melting did not seem to be quite as complete, round melt zones were visible, very closely spaced in an homogeneous way throughout the treated surface. In low magnifications, this morphological pattern of laser beam-dental mineral interaction had a characteristic "honeycomb" appearance. At fluences 0.5 and 0.4 J/cm^2 the round melt zones were still visible but much smaller than at 0.6 J/cm^2, with larger intervening areas of apparently unaltered enamel. At 0.3 J/cm^2 the irradiated surface was barely affected by the laser light (Figs. 6 and 7). In low magnifications some faint "honeycomb" pattern was seen, which lacked regularity both across the very same sample and in-between samples. In other words, at 0.3 J/cm^2 some areas of the irradiated surface exhibited minimal but yet definitive alteration of surface morphology as a result of the dental mineral-laser interaction, while others none. Lower fluences did not seem to cause any discernible surface alteration.

At 10.6 μm modification of irradiated surfaces was detectable for fluences 4-2 J/cm^2 (Figs. 8 and 9). For example, at fluence 4 J/cm^2 all samples exhibited an almost complete melting of the irradiated surface. In high magnifications, wavelike structures were also detected but in less numbers and significantly smaller sizes, with a much less dramatic appearance than at 9.6 μm. At fluence 3 J/cm^2 the irradiation effect on the surface morphology was also very prominent but now there were narrow intervening zones of apparently unaltered enamel.

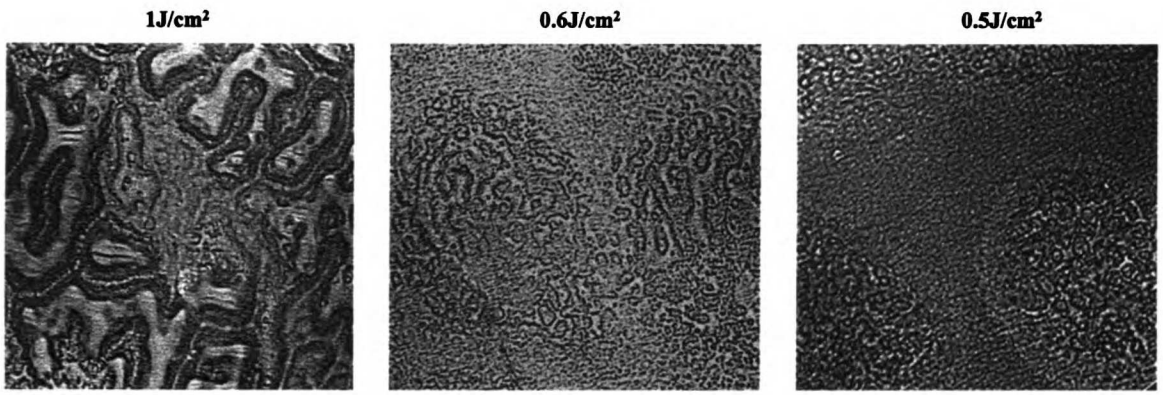

Figure 4. Bovine enamel surfaces after treatment with 9.6 μm laser irradiation at the fluences shown (magnification 500x).

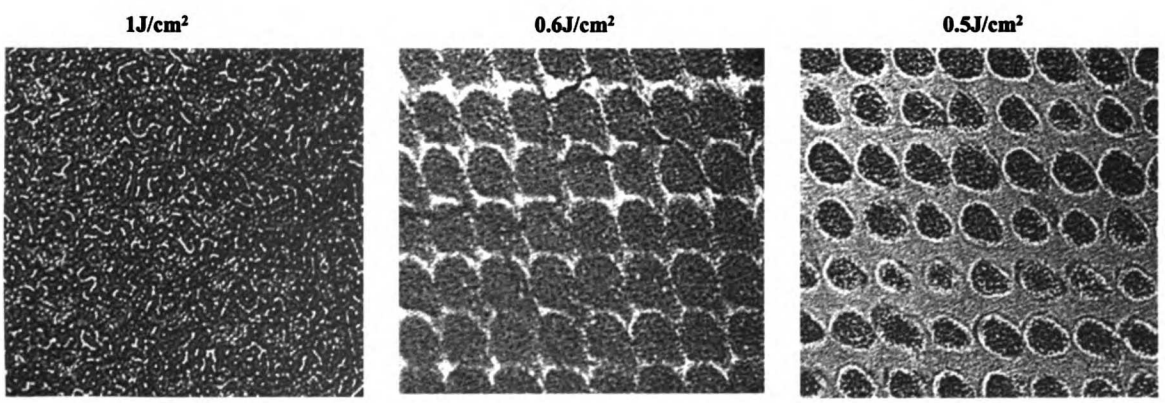

Figure 5. Bovine enamel surfaces after treatment with 9.6 μm laser irradiation at the fluences shown (magnification 100x).

Figure 6. Bovine enamel surfaces after irradiation with 9.6 μm laser beam at the fluences shown, and untreated control (magnification 500x).

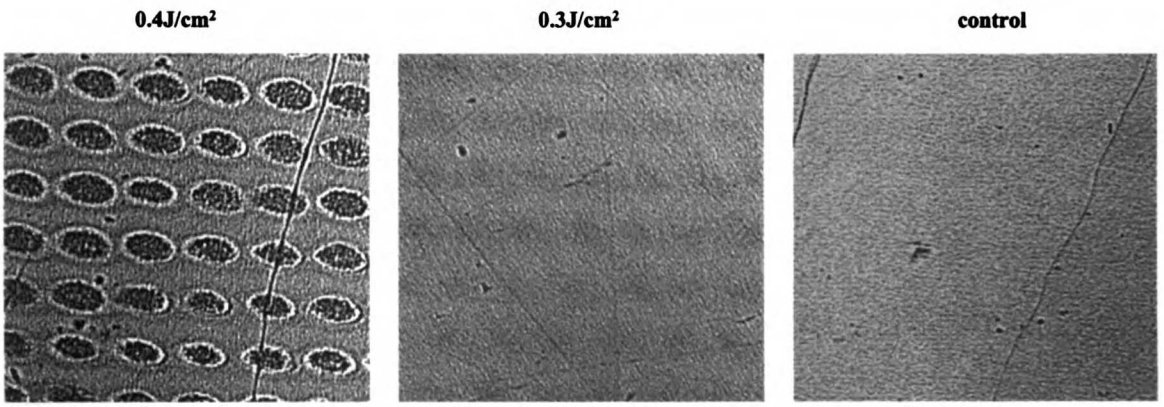

Figure 7. Bovine enamel surfaces after irradiation with 9.6 μm laser beam at the fluences shown, and untreated control (magnification 100x).

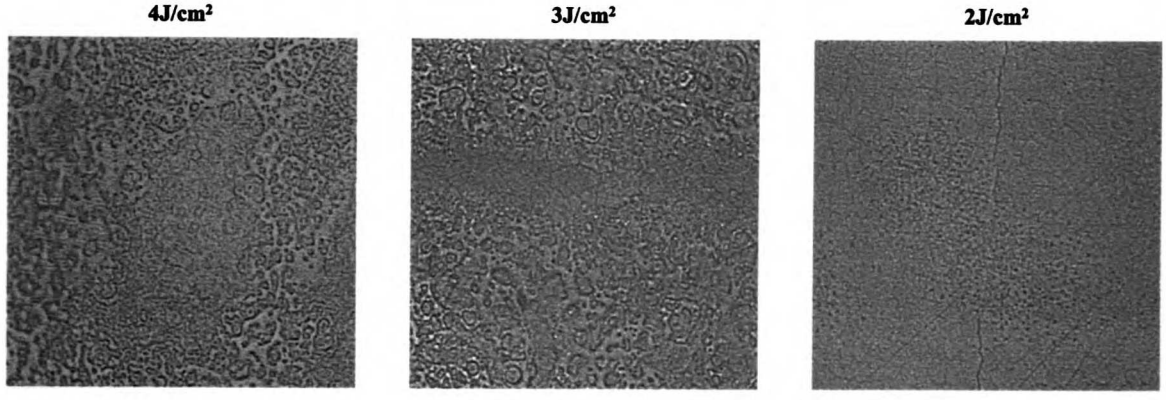

Figure 8. Bovine enamel surfaces after treatment with 10.6 μm laser irradiation at the fluences shown (magnification 500x).

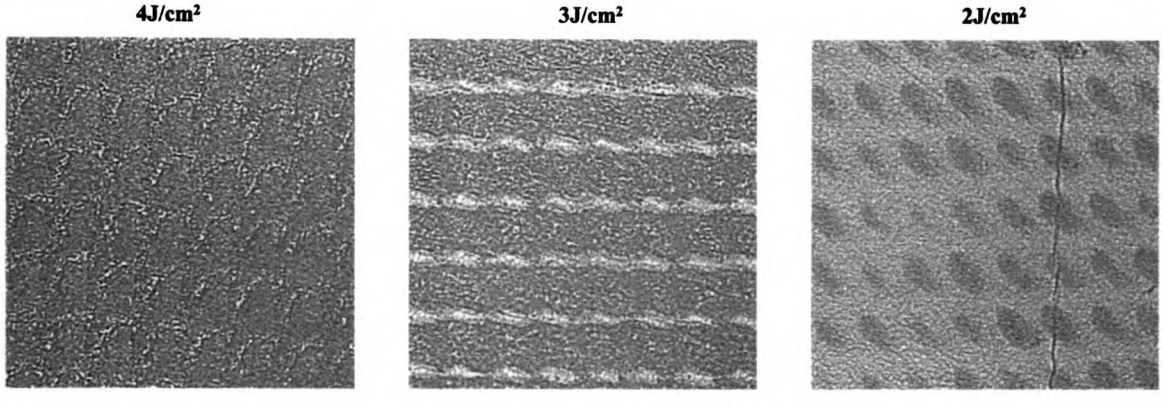

Figure 9. Bovine enamel surfaces after treatment with 10.6 μm laser irradiation at the fluences shown (magnification 100x).

At fluence 2 J/ cm^2 the laser beam did not affect all samples, and even in the same sample not all areas were affected in a similar manner. Ovoid rather than circular areas of morphologically altered surface of varying size were dispersed in-between rather large areas of apparently unaltered enamel. In some samples only a small portion of the surface seemed to include spots of altered morphology, while a large portion of the surface seemed to have no evidence of surface change whatsoever. Lower fluences did not seem to cause any demonstrable surface alteration.

Interestingly, although the dimensions of the modified zones followed the reduction of the fluence within the same wavelength, in all occasions the distance between the centers of these zones remained stable (roughly corresponding to the 200μm distance between successive scan steps of the irradiation procedure). Most probably, these round melt zones correspond to the center of the gaussian-shaped laser beam, where the energy intensity was the highest.

D.3 ACID DISSOLUTION RATE MEASUREMENTS

The amount of calcium and phosphate ions that dissolved into the acid buffer, as determined by atomic absorption and UV-spectroscopy respectively, were plotted in parts per million (ppm) versus time (minutes). The slopes of these plots, calculated by linear regression, represent the initial surface dissolution rates. Steeper slopes indicate higher dissolution rates. Lower slopes indicate increased resistance to acid dissolution. Examples are displayed in Figures 10 and 11. The numerical values of the mean initial dissolution rates are given in Table 2 and their graphic representation versus fluence is

depicted in a variety of ways in Figures 12-21. The relative change in dissolution rates (normalized to control value as 1.0) as it depends on laser irradiation fluence and wavelength is illustrated in Figures 16 and 17.

When the summary of all effects was considered in the MANOVA test, statistically significant differences were shown in both phosphate and calcium dissolution rates between the groups that were treated with 9.6 μm laser irradiation (including the controls). When individual effects were considered, which are in fact the effects of practical importance, only the samples that were irradiated with 1 J/cm^2 exhibited significant reduction in calcium and phosphate dissolution rates as a result of irradiation ($p<0.0001$). Similarly, when the summary of all effects was considered, some statistically significant differences were shown in the calcium dissolution rates between the groups treated with 10.6 μm laser irradiation (including the controls) but not in the phosphate dissolution rates. When individual effects were considered, none of the groups treated with 10.6 μm exhibited statistically significant reduction in calcium and phosphate dissolution rates as a result of irradiation irrespective of fluence used. The very small size of experimental groups (n=3) unavoidably minimizes the power of the statistical analysis, where in fact there may be an effect that remains undetected.

When the mean dissolution rates were plotted as a function of fluence (Figs. 18-21) for each wavelength, strong trends were evident: Among the groups treated at 9.6 μm, the mean dissolution rates of calcium and phosphate appeared to be reduced in a linear fashion, starting with the lowest fluence (0.1 J/cm^2) used (Figs. 18 and 19). Linear regression analysis indicated that the radiation effect in terms of reduction of dissolution rates was linear immediately at 9.6 μm.

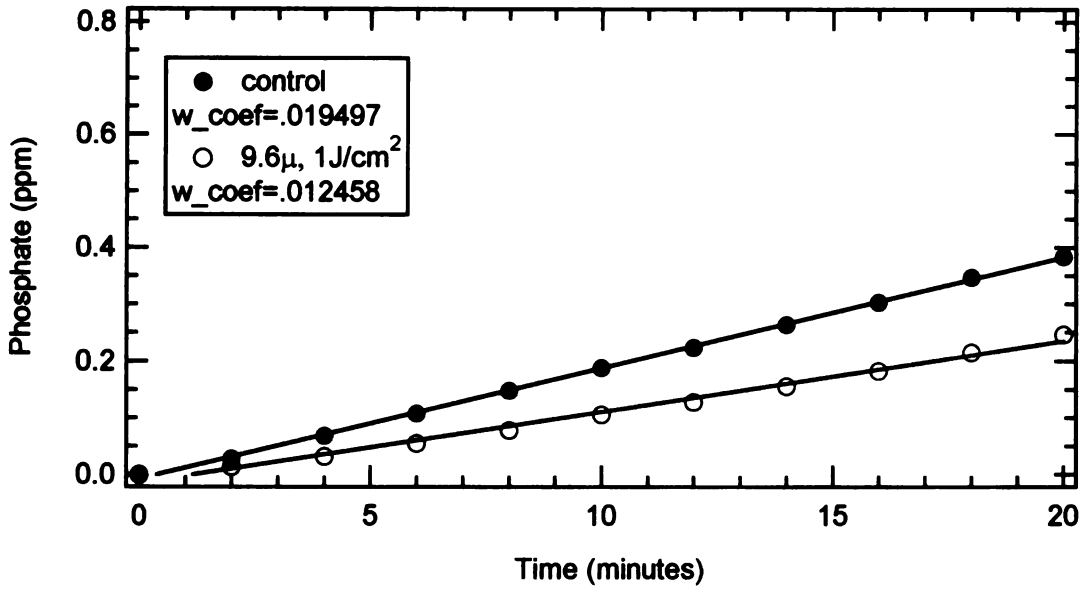

Figure 10. Phosphate dissolving into the acid buffer during the first 20 minutes of acid attack, with and without irradiation of a sample. The slopes (w_coef) represent initial dissolution rates (ppm/min).

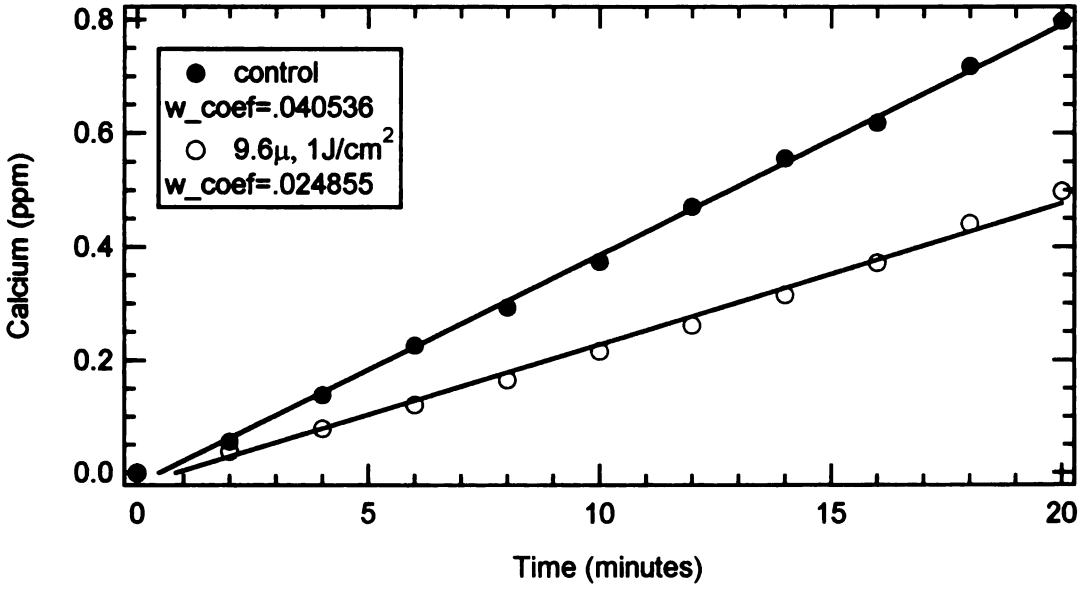

Figure 11. Calcium dissolving into the acid buffer during the first 20 minutes of acid attack, with and without irradiation of a sample. The slopes (w_coef) represent initial dissolution rates (ppm/min).

Table 2. Mean dissolution rates for each of the test groups. Values with * have statistically significant difference from non-irradiated controls ($p<0.0001$).

GROUP	CALCIUM DISSOLUTION RATE			PHOSPHATE DISSOLUTION RATE		
	(ppm/min)			(ppm/min)		
	Mean	SD	% reduction	Mean	SD	% reduction
9.6 μm						
1.0 J/cm^2	0.028851*	0.003163	37	0.015262*	0.001508	32
0.6 J/cm^2	0.033011	0.001492	28	0.016844	0.000887	25
0.5 J/cm^2	0.034376	0.00238	25	0.017359	0.001449	23
0.4 J/cm^2	0.038109	0.002785	17	0.019181	0.00154	15
0.3 J/cm^2	0.040586	0.005565	12	0.020179	0.00213	10
0.2 J/cm^2	0.038808	0.004692	16	0.020674	0.003392	8
0.1 J/cm^2	0.040973	0.007523	11	0.020947	0.004739	7
0.0 J/cm^2	0.045935	0.005825	0	0.022536	0.002891	0
10.6 μm						
4.0 J/cm^2	0.020621	0.006256	47	0.010806	0.002696	47
3.0 J/cm^2	0.027302	0.007175	30	0.014061	0.002843	31
2.0 J/cm^2	0.034011	0.007022	13	0.017055	0.00364	17
1.6 J/cm^2	0.041417	0.001949	0	0.019587	0.001331	0
1.2 J/cm^2	0.038282	NA	0	0.01948	NA	0
0.8 J/cm^2	0.039242	0.005185	0	0.018556	0.003379	0
0.4 J/cm^2	0.038371	0.006831	0	0.018251	0.004692	0
0.0 J/cm^2	0.039275	0.009805	0	0.020483	0.005238	0

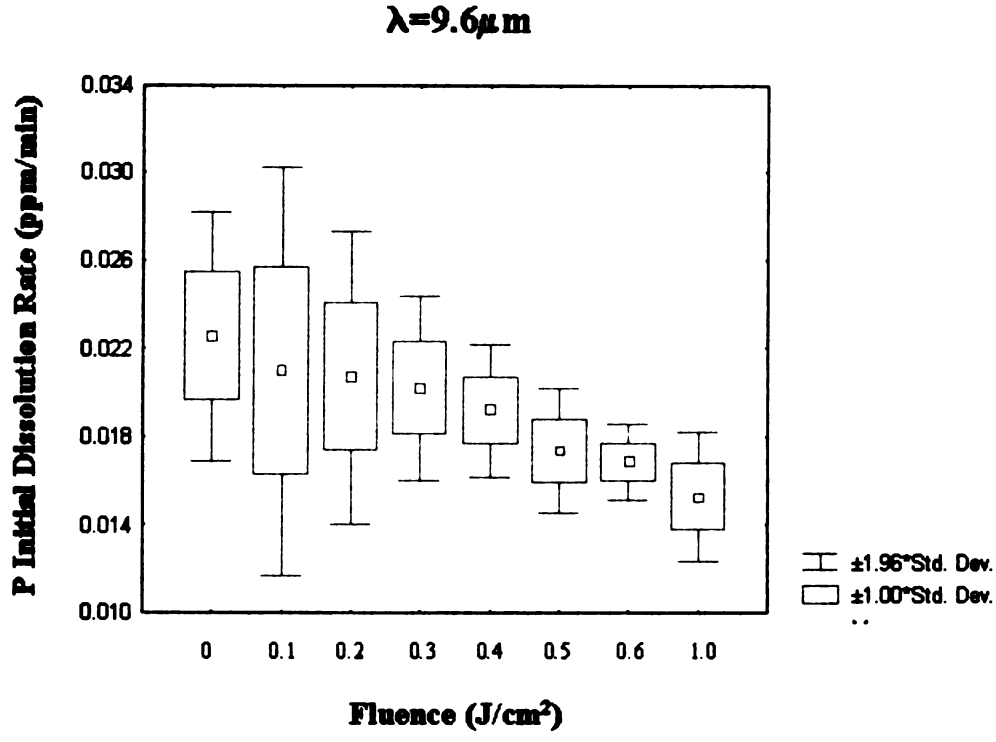

Figure 12. Mean phosphate dissolution rates (small squares) as they correspond to the shown fluence levels at wavelength 9.6 μm.

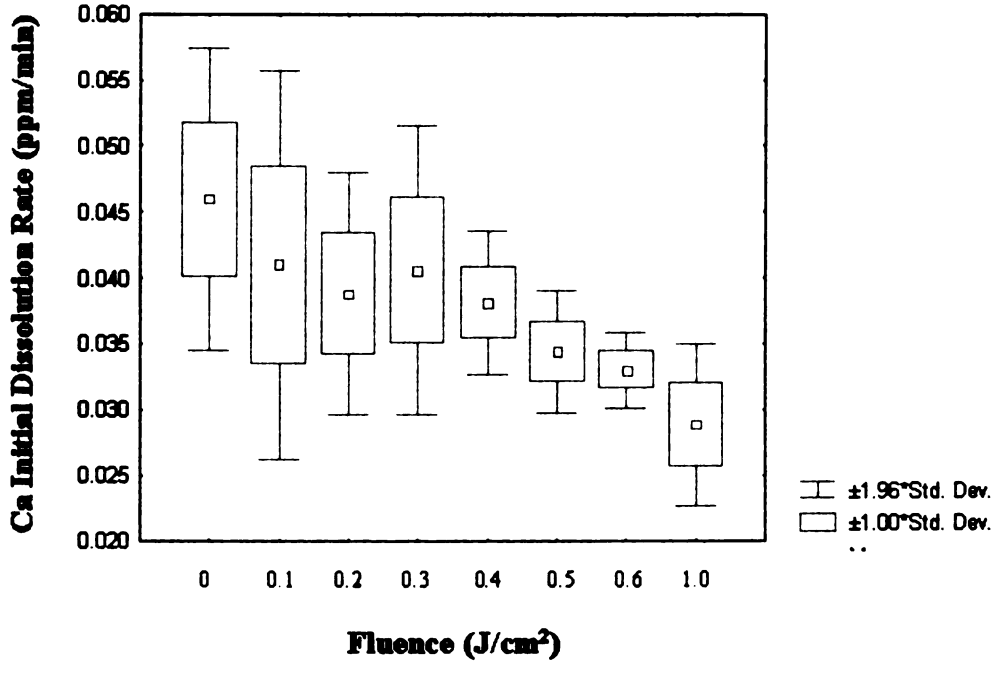

Figure 13. Mean calcium dissolution rates (small squares) as they correspond to the shown fluence levels at wavelength 9.6 μm

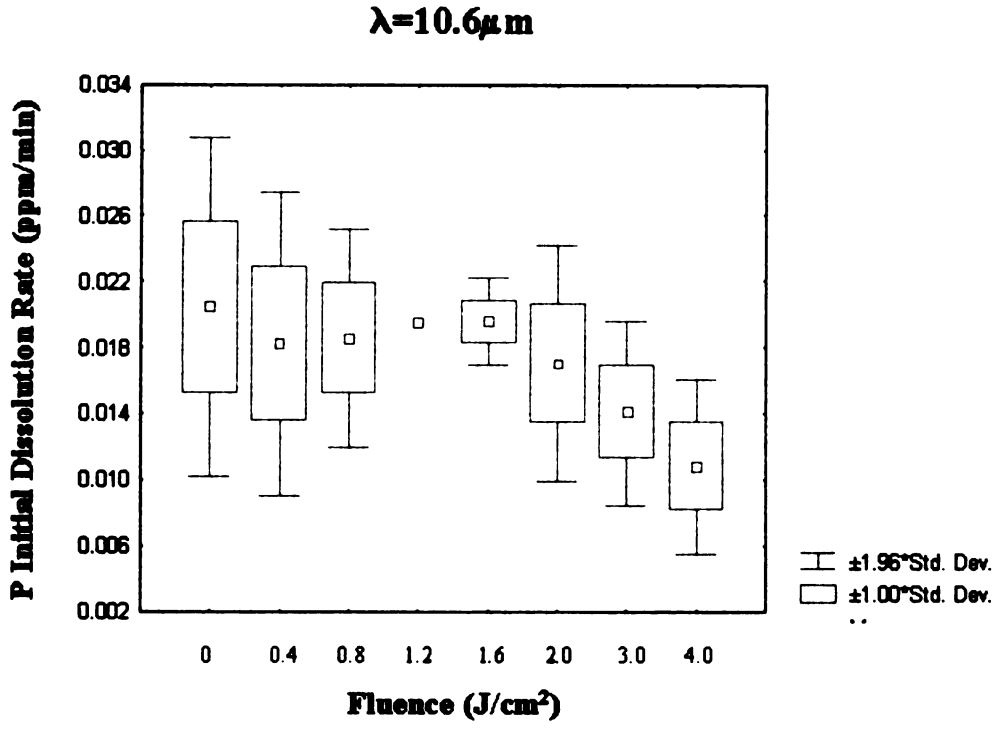

Figure 14. Mean phosphate dissolution rates (small squares) as they correspond to the shown fluence levels at wavelength 10.6 μm. At fluence 1.2 J/cm² only one sample was available for evaluation.

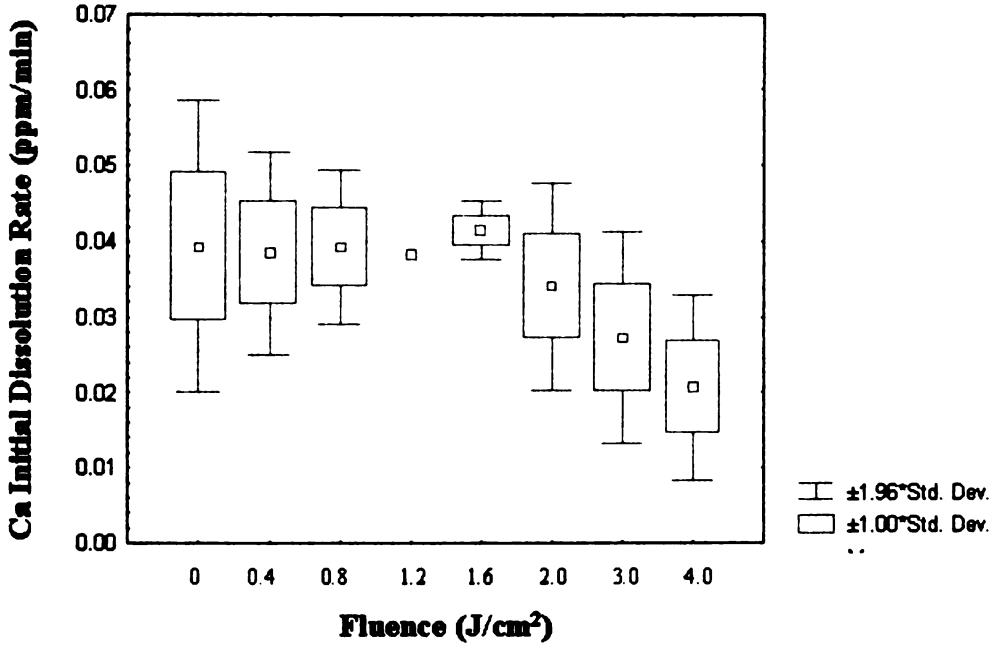

Figure 15. Mean calcium dissolution rates (small squares) as they correspond to the shown fluence levels at wavelength 10.6 μm. At fluence 1.2 J/cm² only one sample was available for

evaluation.

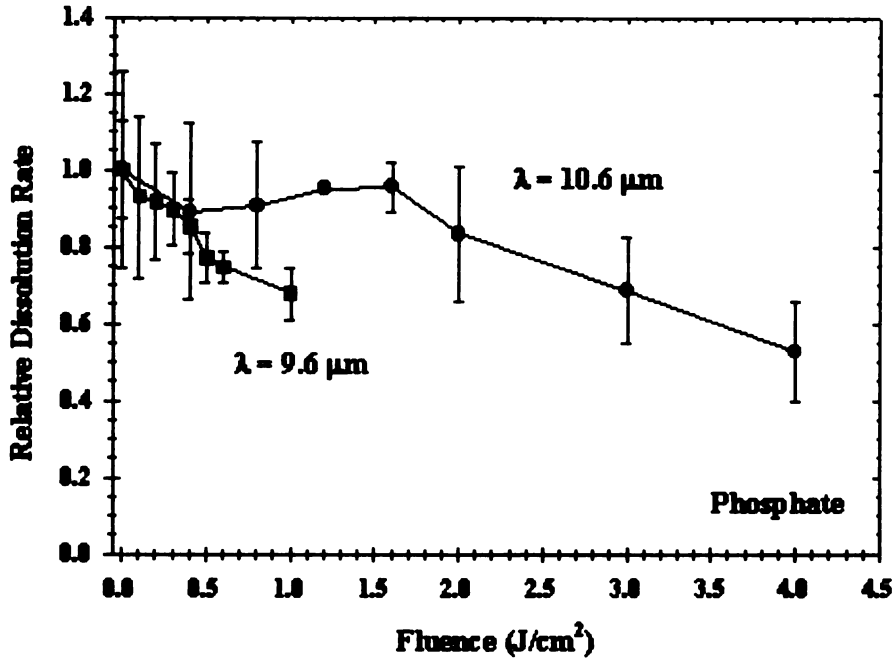

Figure 16. Effect of fluence and wavelength on relative phosphate dissolution rates. Markers represent mean values and error bars standard deviations. (Data is normalized to control mean value as 1.0.)

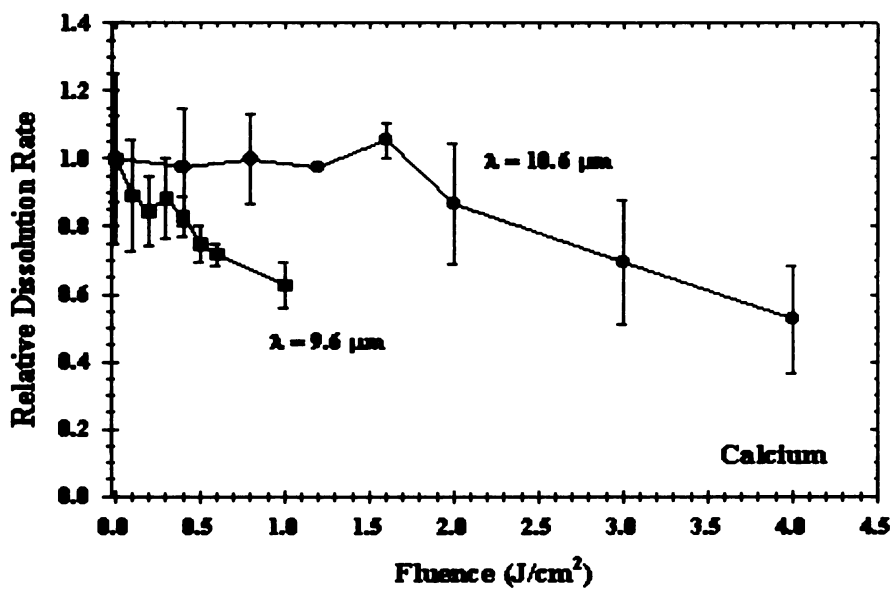

Figure 17. Effect of fluence and wavelength on relative calcium dissolution rates. Markers represent mean values and error bars standard deviations. (Data is normalized to control mean value as 1.0.)

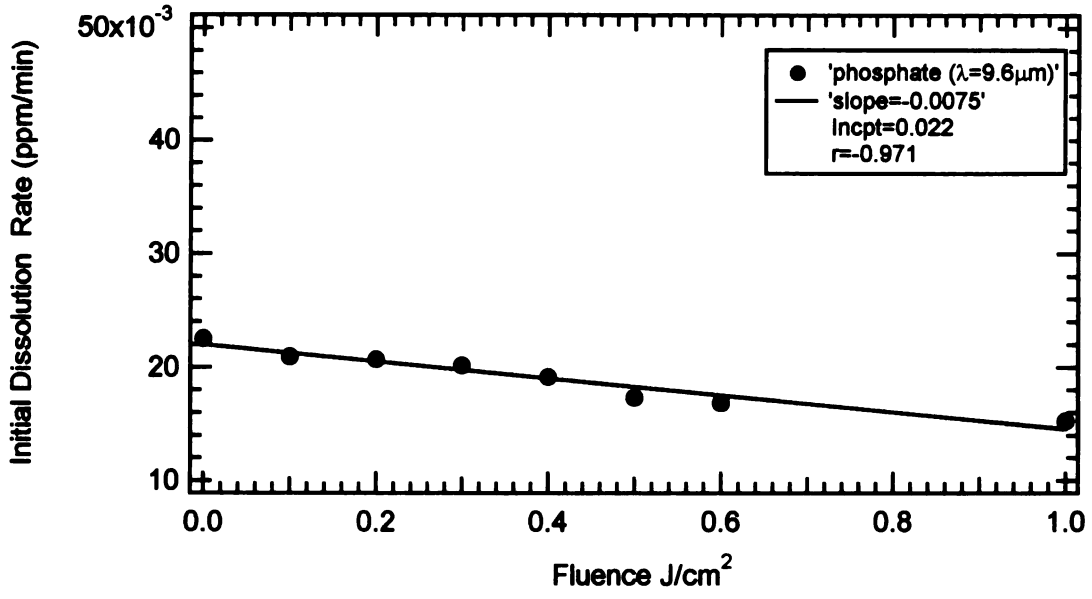

Figure 18. Linear regression plot of mean phosphate dissolution rates as a function of fluence at 9.6 μm (r=linear correlation coefficient; incpt=y axis intercept)).

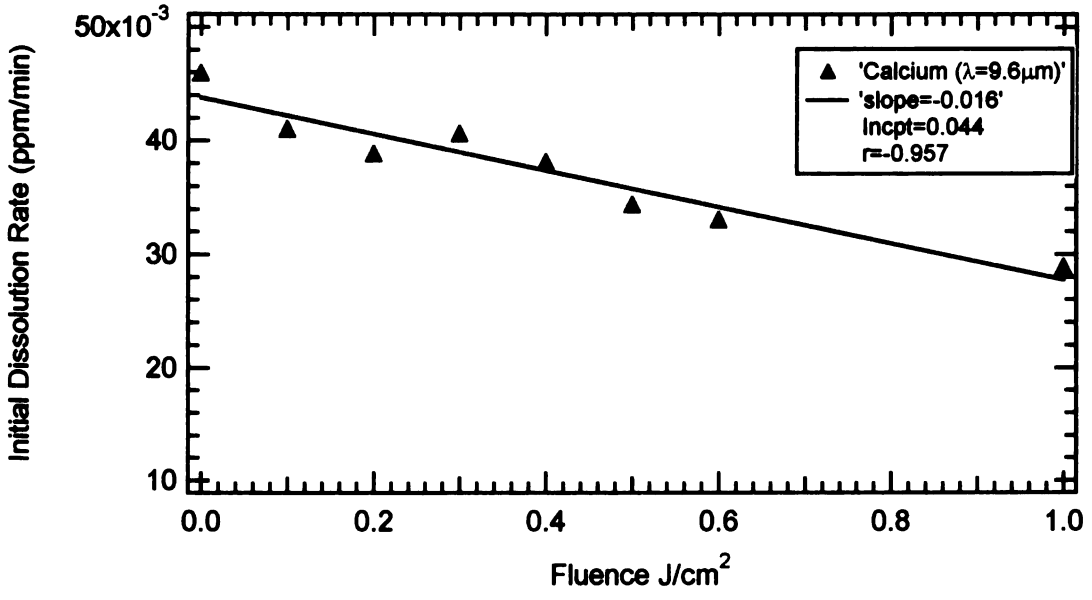

Figure 19. Linear regression plot of mean calcium dissolution rates at 9.6 μm (r=linear correlation coefficient; incpt=y axis intercept).

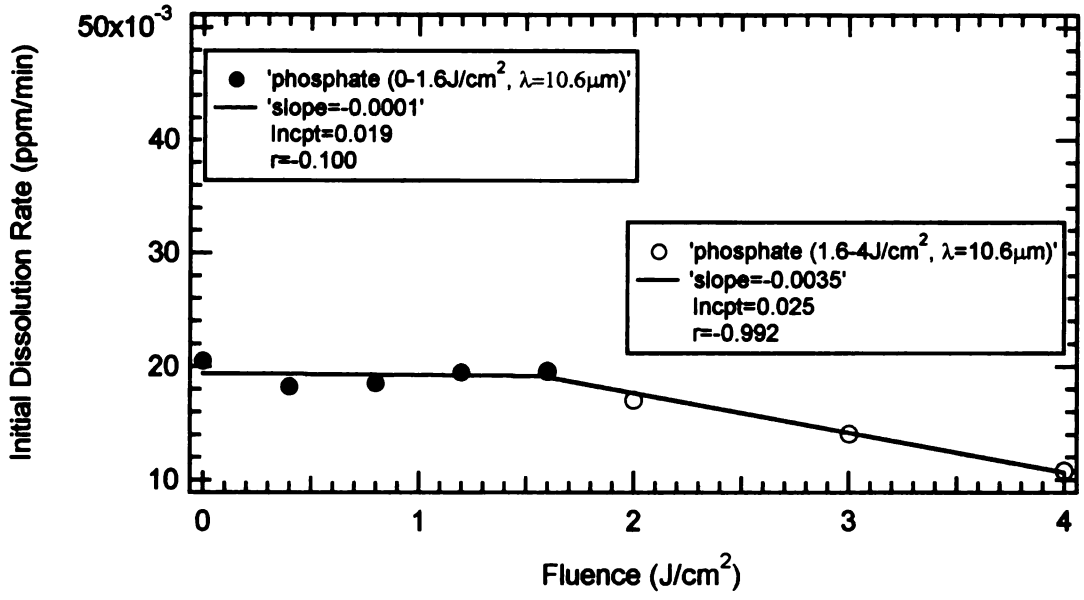

Figure 20. Linear regression plot of mean phosphate dissolution rates as a function of fluence at 10.6 μm (r=linear correlation coefficient; incpt= y axis intercept).

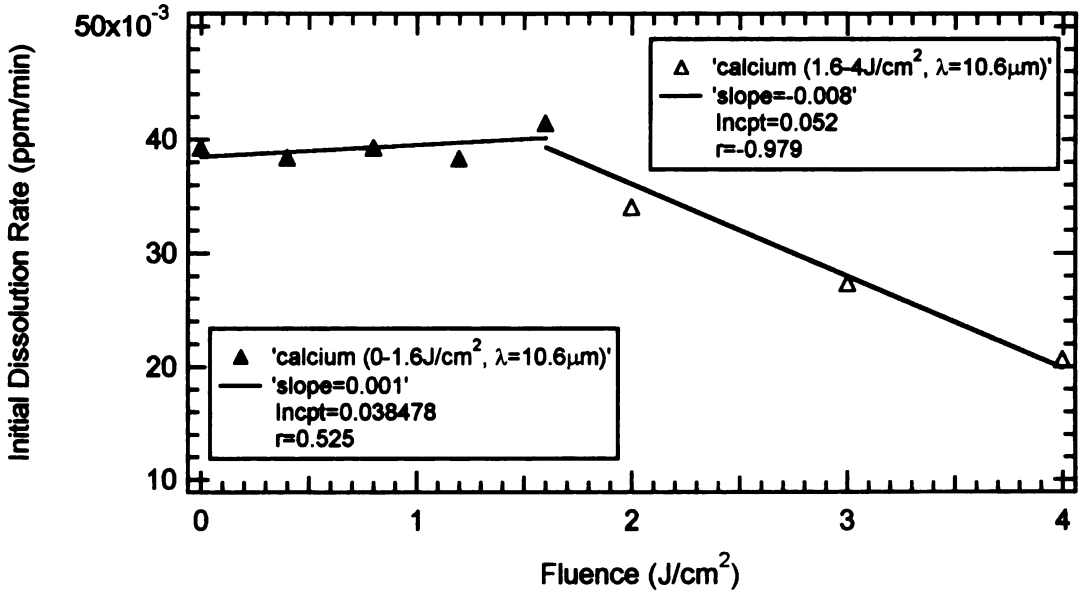

Figure 21. Linear regression plot of mean calcium dissolution rates as a function of fluence at 10.6 μm (r=linear correlation coefficient; incpt= y axis intercept).

All raw data from the controls up to the 1 J/cm^2 group was fitted by linear regression, with correlation coefficients (r) close to +/-1 (-0.957 and -0.971 for the calcium and phosphate respectively), indicating an excellent correlation (Figs. 18 and 19).

A similar trend for obvious and progressive reduction of dissolution rates was evident for the 2, 3, and 4 J/cm^2 groups at 10.6 μm (Figs. 20 and 21). In contrast to the 9.6 μm however, regression analysis indicated that the irradiation effect in terms of reduction of dissolution rates was not linear immediately for treatments at 10.6 μm. Raw data from the 1.6 J/cm^2 groups and above were fitted by linear regression with good linear correlation coefficients (r), (-0.979 and -0.992 for calcium and phosphate respectively). However, data from the non-irradiated controls up to the 1.6 J/cm^2 group showed a poor linear correlation coefficient and a slope effectively zero. In practical terms, it seems that irradiation at 10.6 μm had no effect at fluences below 1.6 J/cm^2, while above 1.6 J/cm^2 a linearly progressive reduction in dissolution rates was implemented.

When percent reductions in dissolution rate mean values were calculated for each group as compared to the control group, the rate of acid dissolution of enamel decreased by 25-37% after treatment with 9.6 μm laser irradiation and incident fluences of 0.5-1 J/cm^2. The reduction in enamel dissolution rates after treatment with 10.6 μm and incident fluences 3-4J /cm^2 ranged between 30 and 47% (Table 2).

E. DISCUSSION

Irradiation of bovine enamel at 9.6 μm with a 2 μs pulsed TEA CO_2 laser produced microscopically discernible surface changes at incident fluences between 0.3-1 J/cm^2. The extent of this effect differed dramatically among the above fluences, ranging from complete surface melt (1 J/cm^2) to a barely observable morphological pattern with partial distribution over the treated surface (0.3 J/cm^2). Similarly, irradiation of bovine enamel at 10.6 μm with the same CO_2 laser resulted in microscopically observable surface changes at incident fluences between 2-4 J/cm^2, ranging from complete melting (4 J/cm^2) to a faintly visible modification (2 J/cm^2). When adjustments were made to account for the energy losses due to reflectance, which is 49% at 9.6 μm and 13.5% at 10.6 μm, it was calculated that the absorbed fluences that produced observable surface effects were as low as about 0.15 J/cm^2 (0.15-0.5J/cm^2) at 9.6 μm, and about 1.7 J/cm^2 (1.7-3.6 J/cm^2) at 10.6 μm. This demonstrated a difference of 6-11 times between the two wavelengths in the amount of absorbed energy required for morphological changes of enamel surface. In addition, the above energies were strikingly lower than the energies required for surface modification in studies that employed CO_2 laser pulses of duration other than 2 μs as is described below.

In the literature, the majority of studies that report surface modification effects on enamel after pulsed CO_2 laser irradiation used irradiation conditions dissimilar to those reported here. The earliest reports involved high numbers of very short pulses (100-200 ns) at very low repetition rates, low fluences per pulse, and high irradiation intensities the reason being: a) mere availability of laser system, and b) lack of knowledge of the

principles of laser-hard tissue interactions. For example, Nelson et al. in the 1980's were the first to report morphological changes in enamel after irradiation with pulsed CO_2 laser light.[52] A total of 400 pulses of 100-200 ns duration each, fluence approximately 0.12 J/cm^2 per pulse, and irradiation intensity about 1 MW/cm^2 were delivered in one spot at a rate of 0.67 Hz. In scanning electron micrographs, changes induced by this irradiation appeared as equally roughened or blistered central regions of about the same size at 9.3 and 9.6 μm, and as smaller less roughened areas at 10.3 μm. No significant surface roughening was detected at 10.6 μm. Irradiation with one-fifth lower total energy produced hardly any surface roughening even at 9.3 and 9.6 μm. Scanning electron microscopy (SEM) at higher magnifications revealed the nature of this surface modification in more detail and indicated a dependency on wavelength as the degree and extent of crystal melting and fusion differed significantly among the different wavelengths (9.3 and 9.6 μm showing the most dramatic effects). We know now that what might have appeared initially as simple wavelength dependent morphological differences, in reality was much more complex. For example, differences in absorbed fluence were unknown and the selected irradiation parameters did not take into account the about 40% higher reflectance at 9.6 than at 10.6 μm. Since the selected incident fluence was the same for all four wavelengths used in the above study, the actually absorbed fluence was much less at 9.6 μm than at 10.6 μm magnifying further the effect of different absorption coefficients. The multiple differences in the irradiation delivery parameters between the two studies render comparisons of the laser-induced morphological changes difficult.

As the understanding of laser-hard tissue interactions advanced, research shifted towards the utilization of different types of pulsed CO_2 lasers capable of producing longer pulses, at higher repetition rates, and higher fluences per pulse. McCormack et al. demonstrated by modification of the irradiation parameters that observable enamel surface changes could be achieved with fewer pulses and much lower total irradiation energy than in the previous study (irradiation intensities<25 kW/cm^2).[93] SEM observations verified the wavelength dependence of these changes. Their experimental approach was more refined, designed to isolate specifically the wavelength effect by keeping all the other parameters identical during comparisons. Surface modification was compared between the four CO_2 wavelengths after 25 pulses of 100 μs duration each, delivered with a frequency of 10 Hz, and with equal absorbed fluences ranging from 2-20 J/cm^2. At 9.3, 9.6 and, to a lesser extent, 10.3 μm rapid surface melting and enamel crystal fusion was observed at absorbed fluences as low as 5 J/cm^2. At 10.6 μm, observable surface melting required absorbed fluences higher than 10 J/cm^2, for these relatively long pulse durations. Another interesting finding of this study was that within each wavelength, at constant, relatively low fluence and number of pulses, a reverse relation was identified between pulse duration and extent of surface modification. For example, at 9.3 μm with a fluence of 5 J/cm^2 per pulse, the surface showed minor melting after 25 pulses of 500 μs duration each, while with the 50 μs pulses the melting was complete, characterized by formation of large octahedral crystals. Such relation was not found at higher fluences that generated surface temperatures well in excess of the melting point of the mineral. These phenomena correlate well with what is known now about the tissue thermal relaxation characteristics, as will be explained later.

Kantorowitz et al. observed, with SEM, similar effects on enamel surface morphology using CO_2 laser irradiation under conditions almost identical to the previous study (100 μs pulse width, 25 pulses, 10 Hz).[66] At 10.6 μm and incident fluence of 12 J/cm^2 (~10J/cm^2 absorbed fluence) little or no morphological changes were evident. In contrast, extensive changes were present at 9.6 μm, with fluences as low as 5 J/cm^2 (~2.5 J/cm absorbed fluence), characterized mainly by crystal fusion and some exfoliation. A very interesting finding of this study was the lack of correlation between caries resistance and enamel surface morphological changes. A significant inhibitory effect on caries-like lesion progression was detected in surfaces with no morphological changes. In fact, the inhibition exhibited at 10.6 μm, where no signs of fusion were identified, was greater than at 9.6 μm, where extensive melting and fusion was observed (87% vs 59%). There are several theories in relation to the potential mechanism of caries inhibition by laser irradiation. They range from the formation of a physical seal due to surface melting and partial fusion to just alteration of the individual enamel crystals to a more acid resistant composition. The findings of the above study support the theory that laser-induced caries inhibition is mainly due to compositional changes of the enamel mineral.

Featherstone et al., in SEM observations, found that discernible surface changes characterized by generalized surface melting and early fusion could be achieved at 9.3 and 9.6 μm with incident fluences as low as 2.5 J/cm^2 after 25 pulses of 100 μs each, and repetition rate of 25 Hz.[125] The same fluence, with a pulse width of 500 μs, limited the morphological changes only to the surfaces treated at 9.3 μm. At 10.6 μm, a fluence of 5 J/cm^2 and either 100 μs or 500 μs pulses were required to produce some limited surface effect, characterized by only small melting zones and mostly crystals of original size.

When the inhibitory effects on caries-like lesion progression were evaluated, it appeared that pronounced surface heating and crystal fusion was not necessarily the most beneficial. Rather, some limited surface melting in its early stages produced a trend to better inhibition. This result indicated, as the previous study, that compositional changes induced by CO_2 laser irradiation, which may or may not be associated with surface morphological changes, are more important for caries inhibitory effects than surface sealing by recrystallization. In fact, there may be a fine balance between morphological and compositional changes, which when achieved confers the highest caries inhibitory effect on irradiated enamel surfaces.

With advancing knowledge, research shifted towards the use of shorter pulse durations closer to the thermal relaxation time of the tissue, as in the present study, with the expectation to use much lower fluences to produce similar effects in terms of surface modification and caries inhibition. Zuerlein et al. reported on the modification of enamel surface morphology induced by irradiation at 9.6 μm with 1-50 pulses of 2 μs each, delivered at a rate of 0.5 Hz, with a fluence of 2 J/cm^2 per pulse.[69] Melting was evident even after a single pulse. With advancing number of pulses, the extent of melting increased greatly, having an appearance very similar to the one noted in the present study for 1 J/cm^2 and 5 pulses delivered at 1 Hz. Similar wavelike structures were observed, attributed to rapid vaporization of the mineral and spallation of molten enamel due to laser-generated stress transients. Zuerlein et al., using experimental measurements and mathematical modeling, calculated with high accuracy the absorption coefficients of enamel for each CO_2 wavelength.[15,69] It is these absorption coefficients which determine the thermal relaxation times of the irradiated tissue, which in turn influence the

distribution of temperature inside the tissue and the overall heat loading. For the highly absorbed 9.6 μm laser light the primary determinant of the melting threshold of enamel would be expected to be the pulse duration. In contrast, for the more weakly absorbed 10.6 μm light the melting threshold would be mostly determined by the thermal relaxation time (i.e., the absorption coefficient).

The melting thresholds reported in the previously cited studies are in accordance with the optical properties of enamel as they were calculated by Zuerlein et al.[15,69] For example, the relatively high melting threshold fluences found for the 9.6 and 10.6 μm light with a 100 μs pulse duration (2.5 and 5 J/cm^2 incident fluences respectively)[125] are compatible with the explanation that at this pulse duration, which is longer than the thermal relaxation times corresponding to either wavelength, only part of the energy is deposited on the surface while the remainder is carried deeper in the subsurface resulting in inefficient surface heating. In addition, the influence of the tenfold difference in absorption coefficients is attenuated at these longer pulses, as it is reflected by the less dramatic difference in the amount of absorbed energy between the CO_2 wavelengths required for surface modification. When the 2 μs pulse duration is employed with a 9.6 μm beam, which is very close to the thermal relaxation time of enamel at 9.6 μm (~1 μs), a larger portion of deposited energy remains confined at the surface, and that explains the much lower levels of melting threshold fluences (0.3-0.4 J/cm^2 incident fluence) as compared to the 100 μs pulse. At 10.6 μm, utilization of 2 μs pulses, which are shorter than the thermal relaxation time of the tissue at this wavelength (~90μs), also lowers the melting fluence threshold (2-3 J/cm^2 incident fluence) in comparison to the 100 μs pulses. This reduction is much less dramatic than at 9.6 μm because at this wavelength the

absorption coefficient is an order of magnitude lower and the relative influence of pulse duration on heat distribution is minimized compared to 9.6 μm.

As has already been mentioned, beneficial changes in acid resistance of dental enamel after CO_2 laser irradiation were observed early on in the literature at temperatures below the melting point of the tooth mineral. Featherstone, Fried, and coworkers hypothesized that this action of laser is due to heating of the enamel mineral at and immediately below the surface to temperatures 400°C and above, which drives the carbonate off the crystals producing a lower solubility mineral with increased resistance to acid induced demineralization. In order to gather direct evidence to support this suggested mechanism, Featherstone et al. used specular reflectance FTIR to determine in a nondestructive way the chemical changes induced on bovine enamel surfaces by pulsed CO_2 laser irradiation at 9.3, 9.6, 10.3, or 10.6 μm, 100 μs pulse duration, 25 pulses per spot at 10 Hz, and incident fluences 0-6 J/cm^2.[68] Loss of carbonate was found to be wavelength and fluence dependent. At 9.6 μm (and 9.3 μm), progressive loss of carbonate with increasing fluence resulted in complete disappearance of the carbonate bands centered at 6-7 μm at an incident fluence of 4 J/cm^2 or greater. At 10.6 μm, the carbonate band disappeared at fluence levels of 5-6 J/cm^2. When reflectivity was taken into account, it became evident that about twice the absorbed fluence was required at 10.6 μm to achieve the same results as at 9.6 μm (and 9.3 μm). These findings correlated well to previous data from *in vitro* studies evaluating the inhibitory effect of CO_2 laser irradiation on the progression of artificial caries-like lesions of dental enamel (i.e.; subsurface dissolution) in relation to wavelength and fluence.[13,14] In those studies, optimum protection against caries progression coincided with incident fluences of 4-5

J/cm^2 at a wavelength of 9.6 μm, utilizing 25 pulses in each spot, with a repetition rate of 10 Hz, and a pulse duration of 100 μs.

It was known from older conventional heating studies that at temperatures between 400-600°C about 30% loss of carbonate takes place, with the remainder slowly removed with advancing temperatures.[61] When Fried et al. assessed the surface temperatures generated by irradiation of enamel under similar laser conditions as the above-mentioned, using time-resolved radiometry, their findings indicated a peak surface temperature range at 9.6 μm between 250 to 1400°C at incident fluences 1 to 6 J/cm^2 respectively.[75] The peak surface temperature achieved at 4 J/cm^2, where total carbonate loss was first observed by FTIR, was found to be approximately 800°C, indicating that most probably this is the temperature that needs to be reached before complete elimination of carbonate is seen.

In order to complete the picture, Featherstone et al assessed the effect of pulsed CO_2 laser irradiation on the surface dissolution kinetics of enamel in relation to fluences and number of pulses per spot.[20] For these dissolution experiments 0-25 pulses were used per spot, with 100 μs pulse duration, 10 Hz repetition rate, and incident fluences 0-8 J/cm^2 at the highly absorbed wavelength of 9.3 μm. The results indicated maximum inhibition of dissolution at fluences 3-5 J/cm^2, coinciding with advanced loss of carbonate from the carbonated apatite mineral of the irradiated enamel as reported previously.[68] This level of inhibition was reached with as few as 5 pulses per spot, with increasing number of pulses offering only minimal additional benefit. These surface dissolution results were in accordance with results from subsurface demineralization experiments mentioned previously.[13]

In the present study, irradiation at 9.6 μm with 5 pulses of 2 μs duration each and 1 Hz repetition rate, resulted in complete disappearance of the carbonate bands at incident fluences of approximately 0.6-1 J/cm^2. At 10.6 μm, the 2 μs pulses resulted in elimination of the carbonate bands at incident fluence levels of 3-4 J/cm^2. When the two wavelengths are compared in terms of required absorbed fluences for elimination of the carbonate bands, it is evident that the 9.6 μm laser light is much more efficient, requiring 7-8 times less energy for similar results. Within each wavelength, when the energy efficiency is compared between the short (2 μs) and longer pulses (100 μs), it is evident that the energy required for elimination of the carbonate bands at 9.6 μm is 4-7 times lower for the short pulse. At 10.6 μm the short pulse is associated with a less dramatic reduction in energy requirements, yet, it does bring a factor of 1.5-2 difference in the fluence required for carbonate elimination. When surface dissolution rates were determined for the irradiated samples relative to non-irradiated controls, it was found that at 9.6 μm, incident fluences as low as 0.6-1 J/cm^2 were able to produce a reduction of about 30-40%. In fact, the reduction in the relative surface dissolution rates closely paralleled the reduction in the relative carbonate band intensities in a manner very similar to the previously mentioned studies using the longer (100 μs) pulses.[20] When 10.6 μm irradiation was used, it required significantly higher fluences, at the level of 3 J/cm^2, to achieve a similar reduction in the relative dissolution rates. If absorbed energies are considered, this finding translates to a difference of about 6-10 times higher energy requirements for use of the 10.6 μm wavelength with similar results. In terms of statistics, only one set of irradiation conditions demonstrated significant reduction of dissolution rates in comparison to controls (i.e., 9.6 μm, 1 J/cm^2). Statistical analysis in

this case has the severe limitation of lack of power due to the very small number of experimental samples in each group. In this study, we wished to cover numerous steps in a range of fluences, and for that purpose, three samples at each individual point were considered adequate. Large variances were observed and unless additional numbers of samples could be used, meaningful interpretation of the statistics by individual point is difficult. Nevertheless, there is a very clear trend of parallel reduction in the carbonate content and dissolution rates with increasing fluence within each wavelength, for the tested fluence ranges. This trend was clearly shown by the significant linear regression found in each case.

Phan et al. found an almost complete (85%) elimination of carbonate in the FTIR spectra of bovine enamel samples after irradiation by CO_2 at 9.6 μm, 25 pulses per spot, 1 Hz repetition rate, 2 μs pulse duration, 1 J/cm^2 incident fluence, and a very close overlap of the adjacents spots.[49] This was combined with a 70-80% reduction in surface dissolution rates. In comparison to the longer pulses (100 μs) used in the previous studies, the above finding indicates that shorter pulses require fluences 3-5 times lower to achieve a similar reduction in surface dissolution rates (1 vs 3-5 J/cm^2). In the present study, irradiation at 9.6 μm and an incident fluence of 1 J/cm^2 produced a reduction of about 40%. Similar reduction with the longer (100 μs) pulses was produced by an incident fluence of about 0.8 J/cm^2 but the number of pulses per spot (n=25) and the repetition rate (10 Hz) were much higher.[20] It is possible that in the present study, where only 5 pulses were used per spot rather than 25, and a repetition rate of only 1 Hz, irradiation caused removal of carbonate to a depth that was insufficient to produce a higher degree of protection.

Zuerlein et al. found experimentally that the modification depth of laser-treated dental enamel at 9.6 μm was larger for the longer (100 μs) pulses than the shorter (2 μs) ones.[69] After irradiation of bovine enamel at 9.6 μm with 5 pulses per spot of either 2 μs, at 0.5 Hz, or 100 μs, at 1 Hz, and incident fluences of 2 J/cm^2 or 4 J/cm^2 respectively, evaluation of carbonate loss in FTIR spectra as a function of depth showed a progressive decrease in the amount of carbonate loss. This decrease ranged from near completeness at the surface to zero at 6 μm with the short pulse, and at 11 μm depth with the longer pulse. Mathematical modeling predicting the depth in which carbonate loss is initiated for each condition corresponded well to the experimentally determined data. The laser energy at 9.6 μm is deposited to a layer of initial thickness of 1.3 μm and then transferred as heat to the interior of the tooth. Since the 2 μs pulse is very close to the thermal relaxation time of the tissue corresponding to this absorption depth, the heat transfer to deeper layers is minimal, limiting the depth of treatment. In contrast, a pulse of 100 μs duration is much longer than the thermal relaxation time of the tissue, thus permitting additional heat transfer to deeper layers. Therefore, the enamel apparently needs irradiation with a higher number of short pulses for equal modification depth as compared with the longer pulses.

The present study reinforced previous findings pointing towards reduction of the carbonate content of the tooth mineral as the principle mechanism of laser-induced caries inhibition. It also verified the higher energy efficiency of the highly absorbed 9.6 μm laser wavelength in comparison to the less absorbed 10.6 μm wavelength. As shown by optical microscopy, infrared spectrophotometry, and surface acid dissolution experiments, irradiation at 9.6 μm achieved comparable morphological and compositional

enamel surface modification, and increased acid resistance at absorbed fluences several times lower than irradiation at 10.6 μm. Also, this study indicated that within the 9.6 μm wavelength, it might be possible to further reduce the energy needed for enamel caries prevention by using short pulses. The use of short (2 μs) pulses that are very closely matched to the thermal relaxation time of dental enamel for this wavelength produced beneficial morphological, compositional and acid resistance enamel changes at total absorbed energy levels notably lower than the longer pulses of 100 μs duration.

Further experimentation is needed to verify whether, at 9.6 μm, short pulses are more advantageous than longer ones for caries prevention, and also, to find which combination of irradiation conditions establishes optimal caries inhibition. One of the possibilities would be to focus such experiments on identifying the fluences needed to achieve comparable acid resistance, when the only other variable is the pulse duration, and the rest of irradiation parameters remain constant. Changes in carbonate content and acid reactivity (whether surface or subsurface) would depict more accurately the role of the pulse duration if each pulse length was tested at the same range of incident energies per pulse, with identical repetition rates, total number of pulses per spot, laser beam diameter and beam mode, and equal spacing between irradiation spots across the treated enamel surfaces. For example, pulse durations of 2, 8, 50,and 100 μs could be tested for their potential to beneficially alter enamel for caries prevention at 9.6 μm, with a range of 0.5-3 J/cm^2 incident fluences, 5 total pulses per spot, 10 Hz repetition rate, and distances 100-200 μm between irradiation spots to ensure complete coverage of all treated surface. If one of the tested pulse durations were to show a clear energy advantage for similar increases in acid resistance of enamel, more experiments could be performed at this

particular pulse duration. In this subsequent series of experiments, the focus could be shifted towards optimization of performance by manipulation of the total number of pulses and/or repetition rate, while the pulse duration and fluence would be kept constant.

Since there is no single factor solely responsible for the irradiation effects on enamel, it is possible that there is more than one set of conditions at 9.6 μm, which can achieve equally beneficial increase in acid resistance at very similar energy levels. For example, shorter pulses at this wavelength may need lower fluences but higher number of pulses or repetition rates than longer pulses for optimal caries inhibition levels, therefore, negating any significant energy advantages in choosing one pulse duration over the other.

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For reference

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