DENTAL CARIES INHIBITION BY LASER BEAM IRRADIATION
AND CHEMICAL TREATMENT

by

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SUPERVISORY COMMITTEE APPROVAL

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ABSTRACT

The effect of CO₂ laser irradiation on the susceptibility of enamel to dissolution via acid challenge was investigated. A model based on a change in solubility was proposed to explain the observed dissolution rate reduction behavior after human enamel was laser irradiated. This model is consistent with the two-site model which has been proposed to explain the dissolution behavior of precipitated/digested synthetic hydroxyapatite. Laser irradiation reduces the effective driving force for dissolution of HAP from an ion activity product, based on \((\text{Ca})_{10}(\text{PO}_4)_6(\text{OH})_2\), of \(10^{-120}\) to an ion activity product of \(10^{-130}\). Model calculations show that this decrease in driving force will lower the threshold pH for HAP dissolution by about 0.7 pH units.

Temperature profiles during laser irradiation were studied to determine the optimal lasing parameters. Standard heat conduction equations have been modified to account for absorption of energy according to Beer's law and solved using Laplace transforms. The calculated solutions correlate well with surface temperatures measured by infrared pyrometer. The model allows arbitrary combinations of wavelength, pulse pattern, and energy density to be explored. The temperature profile was also correlated well with the laser induced enamel acid resistance assessed by X-ray microradiography.

The additive effect of laser irradiation and chemical inhibitors on the dissolution rate reduction of dental enamel has been demonstrated. The effects of ethane-1-hydroxy-1,1-diphosphonic acid (EHDP), fluoride (F), and dodecylamine HCl (DAC) at various levels upon both the initial dissolution rate (IDR) and mineral density profiles were determined. After laser treatment at higher energies (130 J/cm²), the effects of DAC and/or EHDP are even more dramatic. No dissolution is observed via quantitative microradiography even
after 24 hr of exposure to a sink buffer solution. Such a solution removes about 150 microns of enamel from unlased control samples in the absence of these agents.
To my parents
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CHAPTER 1

INTRODUCTION

Dental decay, or dental caries, is one of the most common oral diseases in the world. Almost everyone has experienced dental caries in his or her lifetime. It has been estimated that it would cost more than 10 billion dollars annually to treat all the dental caries in the United States population (Rowe, 1977). Therefore, development of an effective means of caries prevention would have great social and economic impact.

"What is the mechanism for caries formation?" is the question that has to be answered before any caries prevention method can be developed. It has long been believed that dental caries are initiated by bacteria (Miller, 1884). The most common bacteria are *Streptococcus mutans*, *Streptococcus sanguis*, *lactobacilli*, and other related types. These bacteria adhere to the tooth surface and form a yellowish film called "plaque." The organic acids produced by these oral bacteria can lower the local pH of the plaque to levels at which the concentrations of calcium and phosphates in the environment no longer saturate the tooth mineral (Stephan, 1940). This caries process can be described by the following two simple conceptual equations (Nikiforuk, 1985):

\[
\text{Bacteria} + \text{Substrate} \rightarrow \text{Organic Acids} \quad \text{(Eq. 1.1)}
\]

\[
\text{Organic Acids} + \text{Tooth Mineral (HAP)} \rightarrow \text{Mineral loss (Caries)} \quad \text{(Eq. 1.2)}
\]
According to the above models, caries are the product of the interplay of three factors: the bacteria, the substrate, and the tooth itself. Attempts to reduce caries by non-preventive approaches - for example, better dentists - result in more fillings, but they do not in themselves reduce the prevalence of dental caries (Brown, 1982). Clearly, prevention is the best response to this public health problem (Gift et al., 1983).

"What are the strategies for dental caries prevention?" Based on the three factors identified, three caries prevention strategies have been developed by dental researchers:

1. Eliminate cariogenic bacteria: methods include mechanical plaque removal, chemotherapy (Loe et al., 1976), and vaccination (Bowen et al., 1976; Hamada et al., 1980).

2. Reduce the amount of suitable local substrate for bacteria: methods include dietary control (Bender, 1984) and sugar substitutes.

3. Enhance enamel acid resistance: methods include dental sealants (American Dental Association, 1981), fluoride (Featherstone, 1987), and Laser (Stern et al., 1965).

Fluoride, among all the prevention methods, was thought to be the most effective since it not only inhibits the acid production of bacteria but also enhances remineralization and/or inhibits demineralization. It has been concluded that the use of fluoride has caused a dramatic decline in caries prevalence in the Western world (Featherstone, 1987). Another advantage of fluoride is that it can be conveniently applied in water without interfering with established dietary or oral hygiene patterns.

"Is fluoride the total answer to caries?" It is believed that by using a combination of water fluoridation and weekly fluoride mouthrinsing, a caries reduction of 70-80% can be achieved. This remarkable reduction in dental caries clearly leads one to question whether other approaches such as laser treatment, which carries some risks, is still necessary to further reduce caries. However, there are two drawbacks to the use of fluorides:

1. Fluoride has little effect on caries that occur on the highly susceptible occlusal surfaces; and
2. Many people, especially in the Eastern world, are allergic to fluoride. There is also indirect evidence showing that fluoride may be associated with an increased prevalence of cancer in some Asian countries.

The laser treatment was developed because it has the potential to be effectively applied to occlusal surfaces; also, it could be useful in those areas where water fluoridation is not permitted.

"What kind of laser should be used for caries prevention?" is the first question for everybody interested in laser dentistry. In general, the most popular lasers in clinical medicine and surgery are those with high tissue absorption as shown in Figure 1.1. Stern et al (1970) initiated the first attempt to compare the effects of ruby, neodymium, and carbon dioxide lasers on the enamel surface of extracted human teeth. The inhibitory effects of the various kinds of lasers were compared by examining microradiographs of enamel after acid etching experiments. The results indicated that variations in the subsurface demineralization depended on the type of laser used, and that the carbon dioxide (CO₂) laser was apparently better than the others. They also found that enamel lased with a pulsed carbon dioxide laser at an energy density of 10-50 J/cm² had increased dissolution resistance in an in vivo test, with an effect comparable to that of a ruby laser at energy levels of 200-700 Joules/cm² (Stern et al., 1972). This was attributed to the fact that dental enamel behaves like an opaque solid from the standpoint of energy absorption from the CO₂ laser. From FTIR spectra it was found that human dental enamel has an absorption peak near the wavelength of 10.6 microns, which is the characteristic wavelength of the CO₂ laser as shown in Figure 1.2. Thus, it was thought that the CO₂ laser, with further development, might be the most effective for clinical applications.

"What is the appropriate energy density for CO₂ laser treatment?" then became the second question asked by researchers. This question deals with both effectiveness and safety problems. A diagrammatic representation of structural components of the human tooth is shown in Figure 1.3. As the figure shows, the inside of the hard tissues (enamel
Figure 1.1 Commonly used lasers in clinical medicine
Figure 1.2 Transmittance FTIR spectrum for human enamel powder
Figure 1.3 Diagrammatic structure of a human tooth
and dentin) are bounded by soft tissues (pulp). Goldman et al. (1965) first raised the question of laser induced pulpal damage, and it was reported that a temperature rise of more than 10°F would cause permanent damage to the pulp (Zach, 1965). The pulpal response to irradiation of enamel with continuous wave CO2 laser was recently investigated and it was concluded that CO2 laser power densities of approximately 13 to 102 J/cm² could be used to irradiate enamel of teeth without damage to the pulp (Powell et al., 1989). In addition, the possibility of undesirable laser effects on soft tissues, such as mucous membranes, gingiva, and pulpal tissues must also be considered. The surface membranes of cheek, tongue and gingiva can be protected in practice by the application of a rubber dam prior to laser exposure (Stern, 1971). The possibility of direct injury to dental pulp by laser irradiation of enamel surfaces has also been investigated and an energy density of 10-25 J/cm² from a pulsed CO₂ laser was found to cause no significant pulpal damage (Yamamoto and Ooya, 1974; Yamamoto and Sato, 1980; Lenz et al., 1982).

At this stage, CO₂ laser treatment seems to be promising for clinical application with some further improvement. The theme of this thesis is to investigate the mechanism of the laser effect and to suggest approaches for optimizing the use of the CO₂ laser for dental caries prevention. The theory behind the concept of laser treatment of apatite systems (e.g., enamel, HAP, CAP) will be reviewed in Chapter 2.
CHAPTER 2

LITERATURE REVIEW

Introduction

The use of laser irradiation as a means of inhibiting dental caries was first suggested by Stern and Sognnaes in 1972 (Stern et al., 1972). However, the exact mechanism for such a laser effect is still unclear. We will begin our investigation by reviewing the hydroxyapatite dissolution mechanism. Some of the literature reviewed in this chapter may not be directly related to laser application, but is important to an understanding of its mechanism of action.

The two-site model for hydroxyapatite dissolution

The most widely accepted value for the thermodynamic solubility product of hydroxyapatite, \((\text{Ca})_{10}(\text{PO}_4)_6(\text{OH})_2\), prepared at 100°C is about \(10^{-116}\) (Moreno et al., 1968). However, the apparent solubility product \(K_{\text{HAP}}\), as deduced from dissolution kinetics experiments was found to be only \(10^{-125}\) (Wu et al., 1976), where

\[
K_{\text{HAP}} = a^{10}_{\text{Ca}} a^{6}_{\text{PO}_4} a^{2}_{\text{OH}}.
\]

The apparent solubility really represents a threshold concentration below which dissolution proceeds at a perceptible rate. The concept of apparent solubility, which governs the dissolution kinetics, will be used throughout this thesis.

When HAP samples were exposed to high temperature (1200°C for 30 min), the apparent solubility product is even lower (Hodes, 1972), about \(10^{-130}\). These apparent
anomalies were explained in the late 1970s in a series of papers putting forth the two-site model for hydroxyapatite dissolution (Fawzi et al., 1978; Fox et al., 1978; Griffith et al., 1978). In this model, a rate constant and a threshold solution ion activity product above which no dissolution occurred were deduced for each of two dissolution sites on the hydroxyapatite crystal and the following rate law is obeyed:

$$ R = k_2 (C_s^2 - C) + k_1 (C_s^1 - C) $$

where

- $R$ = dissolution rate (ppm/cm²)
- $k_1$ = first order kinetic rate constants multiplied by surface area
- $k_2$ = first order kinetic rate constants multiplied by surface area
- $C$ = microenvironment concentration of HAP solution
- $C_{s1}$ = apparent solubilities associated with crystalline site #1
- $C_{s2}$ = apparent solubilities associated with crystalline site #2

HAP has an ion activity product of around $10^{-120}$ for $C_{s1}$ (site #1) and $10^{-130}$ for $C_{s2}$ (site #2).

...But it should be emphasized that those solubilities are not true solubilities in the thermodynamic sense. Rather they are apparent solubilities that one could deduce assuming solubility-controlled dissolution and extrapolating the dissolution rate versus concentration dependence to zero dissolution rate... (Higuchi et al., p.153, 1984)

Although this model was criticized (Christoffersen et al., 1979) and a dissolution model based on a polynuclear mechanism proposed (Christoffersen et al., 1980), no other model was shown to be capable of explaining initial dissolution rate data over as wide a range of conditions. Solution suspension experiments were later devised to critically test the two site model, and these experiments were found to be in quantitative agreement with predictions from the original model (Higuchi et al., 1984). Subsequently, Brown et al. (1983) noted that the "apparent solubilities" or dissolution thresholds that had been the
subject of controversy were mathematically equivalent to different activation energies for
the two dissolution sites. More recent work has shown that synthetic apatites can be
prepared with a continuum of populations of site #1 and site #2, depending on the
temperature to which the digested precipitated sample is subsequently subjected (Higuchi et
al., 1986).

Two-site model and caries inhibition effect by laser

In the context of two-site model, the important effect of heat treatment is the
preferential elimination of the "more soluble" site #1 and the conversion of two-site HAP to
the "less soluble" one site HAP. High temperature prepared (1200°C) HAP samples show
this similar effect and behave as a "site #2" crystal with an apparent solubility product of
$10^{-13}$.

Most importantly, laser irradiated HAP samples (50W for 400 sec, 13,000J/cm$^2$)
have recently been shown to undergo a similar effect, that is, laser irradiation converts two­
site HAP to the "less soluble" site #2-only HAP (Wong et al., 1990). Laser irradiation can
be applied in the prevention of dental caries and the two site model for HAP may explain
the mechanism of laser action on the enamel, assuming that HAP is an appropriate model
system for the effect of laser on enamel.

Summary

The observation that exposure of hydroxyapatite to high temperature causes the
more labile site #1 to be annealed away, leaving only site #2 along with the reports that
laser irradiation leads to a decrease in acid susceptibility of enamel, led to our current
working hypothesis that "the laser exerts this effect by briefly raising the temperature of
enamel crystallites high enough that this annealing occurs." This thesis is centered around
this hypothesis. The goal of this thesis is to test this hypothesis and show how laser
operating conditions might be optimized. The basic questions deduced from this goal and
the possible strategies will be discussed in the next chapter. Specific literature directly related to each chapter will be reviewed in the beginning of each chapter.
CHAPTER 3

STATEMENT OF THE PROBLEM

The CO₂ laser has been empirically shown to have promise as a treatment for the prevention of dental caries. The absence of an established mechanism of action, however, makes optimizing the use of this technique an expensive, if not impossible proposition. The aims of this thesis are to establish such a mechanism and therefore the means to optimize laser therapy. Specific questions to be addressed are:

1. What is the mechanism for the caries inhibition effect by CO₂ laser? Is it related to a change of the apparent solubility of enamel? Can this mechanism be related to the hydroxyapatite dissolution mechanism (two-site model)? Is hydroxyapatite a good model system for human dental enamel?

2. Is the laser effect caused by heat? Can we optimize this effect by controlling the temperature? How can we control the temperature of enamel to achieve effectiveness of lasing without damaging the underlying soft tissue?

3. Can we improve the laser effect by the use of chemical agents? If so, what is the mechanism for this improvement? Can we relate this effect to the two-site character of hydroxyapatite?

These questions will be addressed in this thesis using the continuous wave CO₂ laser. General strategies utilizing more flexible equipment, such as the free electron laser, can then be developed.
Overview of strategies

Three main questions will be addressed in this thesis. The strategy for answering question 1 requires the study of the dissolution behavior of CO$_2$ laser irradiated human enamel. The dissolution rate reduction of lased (50W, 2sec = 65J/cm$^2$) human enamel can be observed by rotating disk dissolution experiments to see whether the reductions are similar to those resulting from heat treatment of hydroxyapatite. Calcium and phosphate common ion experiments can also be used in this comparison. The temperature achieved by laser irradiation (50W, 2sec = 65J/cm$^2$) is calculated to be lower than that required (1200°C) to change the solubility of HAP. In order to verify that an apparent solubility change can occur at this lower temperature, apatite containing ~2% carbonate (similar to the carbonate content of human enamel) will be used as a model system.

The strategies involved for the second question rely on an understanding of the temperature profile. A theoretical model will be used to calculate temperature profiles under different lasing conditions. Acid challenge experiments will be conducted and each enamel sample after acid attack will be examined by X-ray microradiography. Variations of the results of X-ray microradiography are expected to correlate with different temperatures achieved in the enamel. Preliminary information concerning optimal lasing conditions can be obtained. Although absolute accuracies of calculated temperature profiles will need further experimental verification, the relative temperatures under different lasing conditions can be reliably ranked.

The approach to the third question requires dissolution rate studies in solutions containing chemical inhibitors which are known to inhibit the dissolution of synthetic hydroxyapatite possessing only site #2. A much more severe dissolution test will also be conducted and enamel samples will be exposed to dissolution media for 24 hr. The dissolution results will be measured by quantitative microradiography.
CHAPTER 4

A THEORETICAL STUDY FOR THE EFFECT OF CO₂ LASER IRRADIATION ON ENAMEL DISSOLUTION

Introduction

The use of laser irradiation as a means of inhibiting dental caries was first suggested by Stern and Sognnaes in 1972 (Stern et al., 1972). They found that enamel lased with a pulsed CO₂ laser at an energy density of 10-50 J/cm² had increased dissolution resistance in an in vivo test. Subsequently, similar findings were reported by other investigators (Yamamoto and Ooya, 1974; Yamamoto and Sato, 1980). Recent in vitro studies have shown that a reduction of solubility was also observed with heat-treatment of human dental enamel (Sato, 1983). Maximum dissolution inhibition was observed when human enamel was heated in an electric furnace for 24 hr at 350°C.

Several possible explanations have been proposed to explain the observed inhibition of caries by both laser and heat treatment

1. Permeability change: the surface is sealed by the laser and is less permeable to the diffusion of acid ions (Stern et al., 1966; Lenz et al., 1982).
2. Solubility change: the composition of enamel is altered by laser irradiation in such a way that solubility is reduced (Yamamoto and Sato, 1980; Kuroda and Fowler, 1984; Fowler and Kuroda, 1986; Featherstone et al, 1987). Fowler and Kuroda also reported that CO₂ laser irradiation can convert a small amount of the mineral components in tooth enamel to β- and α-tricalcium phosphate. However, they were not able to explain the enhanced acid resistance of tooth enamel by those
phase changes because the transformed β- and α-tricalcium phosphate have higher solubilities than hydroxyapatite.

3. Microspace change: laser irradiation creates microspaces and the calcium and phosphate ions dissolved by acid etching are trapped in these microspaces in lased enamel, whereas these ions are released out in unlased enamel (Oho and Morioka, 1990).

Work done with synthetic hydroxyapatite (HAP) in our own laboratories supports the hypothesis that laser irradiation exerts its effect by lowering the apparent solubility of enamel. A two-site dissolution model has been proposed by our group to explain the dissolution behavior of precipitated/digested synthetic HAP (Fox et al., 1978; Higuchi et al., 1984). In this model, HAP has an ion activity product of around $10^{-120}$ for site #1 and $10^{-130}$ for site #2. High temperature prepared (1200°C) "TVA" HAP and heat-treated (1200°C for 30 min) HAP samples have been shown to possess only dissolution site #2 (Yamamoto et al., 1986), while the dissolution of non-heat-treated material is dominated by the more labile site #1. These heat treated apatites have been shown to have an ion activity product ($K_{\text{HAP}}$) of about $10^{-130\pm1}$ in acetate buffer solution (Hodes, 1972). CO₂ laser irradiation at very high energies (50W for 400sec =13,000J/cm²) of HAP pellets has been shown to act similarly to heat treatment in annealing away site #1, resulting in dissolution occurring by site #2 only (Wong et al., 1990).

In this chapter we will test the hypothesis that laser irradiation can change the dissolution behavior of human enamel from that governed by site #1 ($K_{\text{HAP}}=10^{-120}$) to that governed by site #2 ($K_{\text{HAP}}=10^{-130}$).

Assuming that conversion to site #2 occurs, several *a priori* predictions can be made about the dissolution kinetics of enamel:

1. The dissolution rate driving force as deduced from rotating disk studies, will be reduced from $K_{\text{HAP}}=10^{-120}$ to $K_{\text{HAP}}=10^{-130}$. In our sink buffer system, a very insensitive test system, this corresponds to a decrease of molar enamel apparent
solubility by about a factor of 5 in synthetic saliva (calculations will be shown later in this chapter).

2. In addition to decreasing the apparent solubility of enamel, this conversion to site #2 will make the enamel much more susceptible to further reductions in dissolution rate due to the presence of calcium and/or phosphate common ion.

3. (will be tested in Chapter 5) Subsurface dissolution behavior should be observed after an acid etching experiment with laser irradiated enamel in a partially saturated buffer system (pK$_{\text{HAP}}$=126, pH=4.5).

4. (will be tested in Chapter 6) EHDP and DAC have been found to completely inhibit the dissolution of heat-treated (site #2) HAP at concentrations of 3 mM while dissolution of non heat treated HAP (site #1 dominated) is little affected by 3 mM levels of either DAC or EHDP (Otsuka et al., 1987; Otsuka et al., 1989). We would therefore expect laser irradiated enamel to also be more responsive to these chemical agents as well.

5. (will be tested in Chapter 6) The relative effectiveness of fluoride ion would also be enhanced, assuming that dissolution behavior in the presence of fluoride is governed by an FAP ion activity product (K$_{\text{FAP}}$) and that its value is reduced in the same manner as is the K$_{\text{HAP}}$ that governs dissolution in the absence of fluoride.

Each of these a priori predictions based on conversion of site #1 to site #2 will be experimentally tested. An affirmative result would provide a basis for more detailed studies of both the underlying mechanism and the optimization of the laser operating parameters.

**Materials and methods**

**Human enamel**

Human enamel was supplied by the Department of Endodontics, Showa University, and the Department of Dental Education, University of Utah. Enamel slabs of the desired
dimensions were cut by a high concentration diamond wafering blade and the surface ground with a fine sandpaper to remove pellicle. Approximately 100 microns of the enamel were removed from the surface.

**Carbonate apatite (C-HAP)**

The procedure described by LeGeros et al. (1971) was used with slight modification. Calculated amounts of hydrated CaHPO$_4$·2H$_2$O and NaHCO$_3$ were mixed with 4 liters of double deionized water in a glass flask. This suspension was then heated to boiling (~95°C at 5000' altitude) for 10 hr. The residue was centrifuged three times and the final residue was dried at 110°C overnight.

**Hydroxyapatite (HAP)**

The procedure described by Avnimelech et al. (1973) was used with modification. Pure phosphoric acid was added to a boiling (95°C) calcium oxide suspension (carbon dioxide free) in a Teflon flask. The resulting precipitate was digested for 2 weeks in the boiling solution. The residue was centrifuged three times and the final residue was dried at 110°C overnight.

**Solutions**

Acetate buffer solutions (0.1M, pH=4.50, ionic strength=0.5) were prepared by mixing calculated quantities of acetic acid, sodium acetate and sodium chloride along with calculated amounts of CaCl$_2$, NaH$_2$PO$_4$ and using HCl or NaOH if necessary to adjust the pH as determined by a digital pH meter (Altex Model 4500). Required amounts of the acid and its salt and the sodium chloride background electrolyte were calculated with a commercially available computer program (EQUIL, MicroMath Scientific Software, Salt Lake City, UT). The measured pH was generally within ±0.01 unit of the calculated pH.
Heat treatment of C-HAP, and HAP

The HAP and C-HAP pellets were placed in a platinum crucible and then positioned in the furnace (Thermolyne 10500 furnace) which was preheated to the desired temperature.

Laser treatment of human enamel, C-HAP, and HAP

The human enamel block and HAP, C-HAP pellets were positioned in a platinum crucible in a covered porcelain container as shown in Figure 4.1. A stream of nitrogen gas that had been bubbled through double distilled water at 25°C was directed from above into the crucible during the laser irradiation. The enamel surface was irradiated by a continuous wave CO$_2$ laser (LaserSonics) with the power set at 50W for 2 sec (65J/cm$^2$) from a distance of 37 cm. The laser beam width was 14 mm diameter at this distance which is slightly greater than the diameter of the samples used for subsequent dissolution tests. The 65 J/cm$^2$ (50W, 2sec) level was used in our experiments because this energy level has been shown to not result in detectable histological damage to teeth and would likely be clinically acceptable (Stem et al., 1969; Hargreaves et al., 1984).

Initial dissolution rate determination via rotating disk technique

A diagram for the experimental set up is shown in Figure 4.2. Laser treated human enamel slabs were mounted in the rotating disk apparatus with paraffin wax. An enamel surface area of 0.159 cm$^2$ was exposed to the demineralization buffer solution. Exact 50 ml of this buffer solution were pipetted into a water jacketed beaker at 30°C. The rotating speed was controlled at 50 rpm for each run. During dissolution 5 ml samples were withdrawn by pipet at suitable intervals and replaced immediately with 5 ml of fresh dissolution medium. The samples were diluted and analyzed. All glassware and sample holders were cleaned to ensure no contamination.

Analytical methods

The samples were analyzed for either calcium or phosphate depending on the common ion in the dissolution medium. Phosphate concentrations were determined according to the
Figure 4.1 Schematic representation of the apparatus for laser irradiation of enamel
Figure 4.2 Schematic representation of the rotating disk dissolution apparatus
The phospho-ammonium molybdate complex formed was reduced by stannous chloride. The absorbance of the resulting color was determined at the end of 15 min at \( \lambda = 720 \text{ nm} \) in a spectrophotometer (Perkin-Elmer Lambda 7). Calcium concentrations were analyzed by the method of Ray Sarker et al. (1967). The o-cresolphthalein complexone (dye) in ammonia/ammonium buffer complexed with calcium. The absorbance of the resulting color from the calcium-dye complex was determined at the end of 15 min at \( \lambda = 565 \text{ nm} \) in a spectrophotometer (Perkin-Elmer Lambda 7).

**Design of solution compositions for common ion studies**

The expected suppression of initial dissolution rates by various common ion concentrations was calculated as follows. It was assumed that dissolution rate would be proportional to an apparent solubility, governed by an ion activity product (IAP) of the form \((\text{Ca})_{10}\text{(PO}_4\text{)}_6\text{(OH)}_2\) while the crystal structure is C-HAP, \((\text{Na})_{0.305}\text{(Ca)}_{9.695}\text{(PO}_4\text{)}_{5.695}\text{(CO}_3\text{)}_{0.305}\text{(OH)}_2\) (Wong et al., 1990). For various assumed values of this IAP ranging from \(10^{-115}\) to \(10^{-133}\), the ratios of dissolution rate in the presence of a specified common ion concentration to that for no common ion were calculated. Calculations were carried out by a commercially available computer program, EQUIL (MicroMath Scientific Software, Salt Lake City, UT 84121). All calculations assumed the presence of 0.10M acetate buffer at pH=4.5 with ionic strength adjusted to 0.5M by addition of NaCl. Based on these calculations and the hypothesis that lasing would change the driving force from an IAP of \(10^{-120}\) to about \(10^{-130}\), calcium concentrations of 3 and 6 mM and phosphate concentrations of 5 and 10 mM were chosen for the common ion studies. The expected dependencies for dissolution rate on calcium and phosphate common ion concentrations are shown in Table 4.1. As the table shows, these concentrations should allow us to experimentally differentiate the \(pK_{\text{HAP}}=130\) case from the \(pK_{\text{HAP}}=120\) case, and also to differentiate both from the control case (no common ion).
Table 4.1 Calculated suppression of initial dissolution rate for common ion experiments

<table>
<thead>
<tr>
<th>pK&lt;sub&gt;HAP&lt;/sub&gt;</th>
<th>(Sink)</th>
<th>(Calcium common ion)</th>
<th>(Phosphate common ion)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 mM Ca</td>
<td>3 mM Ca</td>
<td>6 mM Ca</td>
</tr>
<tr>
<td>115</td>
<td>100%</td>
<td>93.8%</td>
<td>88.2%</td>
</tr>
<tr>
<td>120</td>
<td>100%</td>
<td>88.2%</td>
<td>77.8%</td>
</tr>
<tr>
<td>125</td>
<td>100%</td>
<td>77.2%</td>
<td>59.5%</td>
</tr>
<tr>
<td>127</td>
<td>100%</td>
<td>70.6%</td>
<td>49.7%</td>
</tr>
<tr>
<td>129</td>
<td>100%</td>
<td>62.5%</td>
<td>39.1%</td>
</tr>
<tr>
<td>130</td>
<td>100%</td>
<td>58.9%</td>
<td>34.5%</td>
</tr>
<tr>
<td>131</td>
<td>100%</td>
<td>53.0%</td>
<td>28.8%</td>
</tr>
<tr>
<td>133</td>
<td>100%</td>
<td>42.3%</td>
<td>19.9%</td>
</tr>
<tr>
<td>135</td>
<td>100%</td>
<td>31.9%</td>
<td>13.1%</td>
</tr>
</tbody>
</table>

* For each assumed value of pK<sub>HAP</sub>, these values represent calculated dissolution rate in the common ion solution as a fraction of the rate in the solution with no calcium or phosphate common ion.
Molar solubilities and threshold pH calculations

Molar solubilities and threshold pH calculations were carried out by the same commercially available computer program, EQUIL (MicroMath Scientific Software, Salt Lake City, UT 84121). One detailed calculation will be presented in the Appendix.

Results

Estimate of extent of annealing

Initial dissolution rate (IDR) studies for both lased and unlased enamel using sink buffer (no calcium or phosphate common ion) are shown in Figure 4.3. As can be seen, the lased samples initially have a dissolution rate about 30-40% that of the control. After about 10 min, the dissolution rate increases fairly sharply to become equal to that of the unlased enamel. We interpret this break in the dissolution curve as indicating that the mineral that has been effectively annealed by lasing has dissolved, and the remaining unannealed mineral therefore behaves similarly to unlased enamel. The amount dissolved by the time the break in the dissolution rate occurs is equivalent to about 0.64 microns of annealed enamel or to a broader zone of partially annealed enamel.

One possible interpretation is that the laser irradiation is almost completely absorbed in the first micron or so of enamel and there is therefore a zone of completely annealed enamel of about this magnitude. Alternatively, heat conduction could lead to a considerably broader zone of enamel being effected. The same amount of apparently less soluble mineral could represent a lessened dissolution rate for the outer unit cell of each crystallite. Such a mechanism could operate, for example, if annealing were related to loss of carbonate and this were limited to the outer unit cell simply by no convenient pathway being available for carbonate loss from the interior of a crystallite.

The initial depression of IDR to ~40% of that for unlased enamel is consistent with the rate determining ion activity product being reduced from $10^{-122}$ to $10^{-130}$. Common ion
Figure 4.3 Dissolution of lased and unlased human enamel under sink conditions
(□) lased enamel (65J/cm²); (○) unlased enamel
studies were done to test the hypothesis that this dissolution rate reduction is related to a decrease in the driving force ion activity product rather than to a reduction in crystallite specific surface area or to some other factor leading to a decrease in crystallite accessibility.

For these studies, IDR\textsubscript{s} were measured in enamel samples both in the presence and absence of common ion. All these experiments were done in such a way that both IDR determinations were in the initial dissolution phase where the IDR was presumed to be governed by annealed crystallites. To ensure that this was the case, two pilot experiments were done (each in triplicate) to show that the annealed crystallite kinetics were being obeyed. In these experiments, the IDR was measured in the presence of calcium or phosphate common ion, in sink buffer, and then again in the presence of common ion. As shown in Table 4.2. The first and third runs were essentially the same, indicating that the experiments were completely within the regime of control by annealed crystallites. This is as expected, since each of these runs was 3 min in duration, and the break in dissolution rate occurred only after 10 min exposure to sink buffer, in which the dissolution rate was almost twice as high as in the common ion buffers used during the 2/3 of the exposure time in this pilot study. In Tables 4.2 it is apparent that experimentally determined rates are reproducible, even though the individual values are not determined with great precision for the common ion runs. This is a result of the necessity of conducting all three runs in a given experiment before the annealed enamel has been dissolved away. Because the individual experiments are not statistically different, we will consider all experimental runs under identical solution conditions simultaneously in estimating the dissolution rate for each condition in subsequent use of this data. Thus data from six separate runs are combined in the determination of the slope for each solution condition.
Table 4.2 Initial dissolution rate (IDR) suppression by common ions, "3 run" protocol

<table>
<thead>
<tr>
<th>Expt #</th>
<th>3 mM Ca common ion</th>
<th>5 mM PO4 common ion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IDR² (µg enamel/cm²/min)</td>
<td>IDR (µg enamel/cm²/min)</td>
</tr>
<tr>
<td>Run 1 (common ion)</td>
<td>9.15 ± 0.55</td>
<td>10.8 ± 0.50</td>
</tr>
<tr>
<td>Run 2 (no common ion)</td>
<td>18.4 ± 0.6</td>
<td>18.2 ± 0.25</td>
</tr>
<tr>
<td>Run 3 (common ion)</td>
<td>9.05 ± 0.45</td>
<td>10.45 ± 0.43</td>
</tr>
</tbody>
</table>

1 Mean ± SD of three experiments, enamel molecular weight was calculated by CAP (-2% carbonate) formula - (Na)₀.₃₀₅(Ca)₉.₆₉₅(PO₄)₅.₆₉₅(CO₃)₀.₃₀₅(OH)₂
**Dissolution rate driving force for lased enamel**

Since the pilot IDR studies had demonstrated the validity of the assumption that IDR kinetics were unchanged for the duration of these short experiments, a more extensive set of common ion conditions were used to test the hypothesis that laser irradiation was exerting its effect by an alteration of the driving force ion activity product. In these studies, both lased and unlased samples were studied and dissolution rate suppression by four different common ion conditions (two calcium levels and two phosphate levels) measured for each. The results are summarized in Table 4.3. For each of the above common ion conditions, the dissolution rate governing $pK_{\text{HAP}}$ can be determined as follows. For a given assumed value of $pK_{\text{HAP}}$, the molar solubilities of enamel in sink buffer and in sink buffer containing the common ion are calculated. This calculation is repeated for various values of $pK_{\text{HAP}}$ until the unique value is found that matches the experimentally observed ratio of IDR in the common ion solution to that in the control.

The best fit values for $pK_{\text{HAP}}$ for both lased and unlased enamel were taken to be those values that lead to the best replication of the experimental ratios of common IDR to control IDR. This was done using a nonlinear least squares parameter estimation program (MINSQ, MicroMath, Salt Lake City, Utah). The results of this fitting are also summarized in Table 4.3.

As is apparent from the above data, the laser treatment in all cases reduced the ion activity product governing the dissolution driving force from about $10^{-120}$ to about $10^{-130}$. This is precisely the effect that has been observed when synthetic hydroxyapatite samples are heat treated at 1200°C. Note that not only did lased enamel have a lower dissolution rate than unlased in the absence of common ion, but the lasing additionally led to a greater percent reduction from this lower baseline in the presence of common ion than occurred with unlased enamel.
Table 4.3  Initial dissolution rate results for common ion experiment

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Common ion</th>
<th>IDR(^1) control IDR</th>
<th>pK(_{\text{HAP}})</th>
<th>best fit(^2) pK(_{\text{HAP}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lased</td>
<td>0</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(50W, 2sec)</td>
<td>5mM PO4</td>
<td>58.7±4.2%</td>
<td>128.1 - 130.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10mM PO4</td>
<td>42.7±6.0%</td>
<td>127.8 - 131.1</td>
<td>129.9±0.68 (127.7-132.1)</td>
</tr>
<tr>
<td></td>
<td>3mM Ca</td>
<td>49.5±4.0%</td>
<td>131.0 - 132.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6mM Ca</td>
<td>39.3±4.0%</td>
<td>128.3 - 129.7</td>
<td></td>
</tr>
<tr>
<td>Unlased</td>
<td>0</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 mM PO4</td>
<td>82.4±6.8%</td>
<td>116.8 - 123.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 mM PO4</td>
<td>71.4±5.2%</td>
<td>118.8 - 122.6</td>
<td>121.42±0.46 (119.9-122.8)</td>
</tr>
<tr>
<td></td>
<td>3 mM Ca</td>
<td>87.0±5.8%</td>
<td>116.3 - 123.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 mM Ca</td>
<td>70.2±5.2%</td>
<td>120.8 - 123.8</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Mean ± SD of three experimental runs.

\(^2\) Best fit pK\(_{\text{HAP}}\) was calculated by all the IDR ratio (%), the 95% confidence range was shown in parenthesis.
The dissolution rate by heat treated and laser treated HAP, C-HAP

The 65 J/cm² (50W, 2sec) level used in our experiments has been shown to anneal enamel crystallites to site #2 HAP. Model calculations (Chapter 5) done by us indicate that the temperature rise in the surface was about 400°C and extends to the first 20 microns of enamel. This result is not consistent with the temperature required (1200°C) to anneal site #1 of HAP crystal. Systematic dissolution rate measurements were therefore made on samples that had been heated up to 500°C as shown in Figures 4.4 - 4.7. This low temperature heat treatment is not enough to cause the dissolution rate reduction for HAP. As shown in Figure 4.4, this is consistent with the previously reported finding that a temperature of 1200°C is required to anneal site #1 (Yamamoto et al., 1986). But 400°C is adequate to cause dissolution rate reduction for C-HAP as shown in Figure 4.6. Similar parallel results were observed for low energy laser treatments as shown in Figures 4.5 and 4.7. As can be seen by comparing Figure 4.5 and Figure 4.7, only 130J/cm² (50W, 4 sec) is required to achieve about 1/3 dissolution rate reduction for CAP as compared to the 1,600J/cm² (50W, 50 sec) for HAP. This leads us to believe that for carbonate containing HAP, which is the case for enamel, solubility reduction could occur even at lower temperature. Synthetic carbonate containing apatite (~2% carbonate) would therefore be an appropriate model for human dental enamel.

Molar solubility calculations

The key to the "effectiveness" of laser irradiation is the dramatic increase in resistance to acid challenge resulting from conversion of enamel from two site dissolution kinetics (predominantly site #1, pK_HAP=120) to dissolution via site #2 (pK_HAP=130) only. A more meaningful comparison than solubility products is the comparison of molar solubilities in various media, especially media approximating the composition of saliva or plaque fluid. Some example solubility calculations are shown in Table 4.4 (see Appendix for details), expressed as mmoles hydroxyapatite per liter. Positive numbers indicate solubility; negative figures are therefore indicative of a supersaturated solution.
Figure 4.4 Dissolution profiles of heat treated hydroxyapatite pellets (0% carbonate)

- (O) HAP control
- (△) 300°C for 30 min
- (☆) 500°C for 30 min
- (☐) 200°C for 30 min
- (◇) 400°C for 30 min
Figure 4.5 Dissolution profiles of laser treated hydroxyapatite pellets (0% carbonate)

- (O) HAP control
- (□) 50W for 2 sec
- (△) 50W for 4 sec
- (◇) 50W for 50 sec
Figure 4.6  Dissolution profiles of heat treated carbonate apatite pellets (1.9% carbonate)

(O)  CAP control
(△)  300°C for 30 min
(☆)  400°C for 30 min
(□)  200°C for 30 min
(◇)  500°C for 30 min
Figure 4.7 Dissolution profiles of laser treated carbonate apatite pellets (1.9% carbonate)
(○) CAP control  (□) 50W for 2 sec
(△) 50W for 4 sec  (◇) 50W for 50 sec
The key points from those calculations are that a change of 10 orders of magnitude in the exponent of the apparent solubility product lowers the threshold pH for dissolution by about 0.73 pH units, which corresponds to about a factor of 2 difference in the amount of acetic acid that would need to be added to the saliva for dissolution to commence. At pH 4.5, which corresponds to a moderately severe caries challenge, the anticipated difference in solubilities is about a factor of 5. Although those figures in Table 4.4 are based on a specific set of assumptions regarding saliva composition, other assumptions have been shown to give similar results. The approximately three quarters of a unit difference in the threshold pH for a 10 unit difference in the solubility product exponent holds over a range of assumptions about saliva concentration.

**Discussion**

The dissolution rate reduction for enamel exposed to buffers with no calcium or phosphate common ion is consistent with a reduction of the ion activity product driving force to about $10^{-13}$. Based on only the dissolution rate in common ion free solutions, an alternative explanation for the dissolution rate reduction is that either crystallite specific surface area or enamel permeability is reduced by laser irradiation. SEM data reported by Featherstone (Featherstone and Nelson, 1987) support this explanation for the pulsed laser irradiation used in those studies. SEM examination of both enamel and pellets of CAP exposed to the laser irradiation conditions used in this report failed to show evidence of either melting or significant reduction of crystallite specific surface area. This may be due to the attainment of higher enamel surface temperature with the pulsed laser than with the continuous wave laser used here.

If either surface area or permeability reduction were the only operable mechanism, the IDR suppression effect of common ions (calcium or phosphate) would be the same on both lased and unlased enamel. In each case reported here, the percent of IDR suppression by these ions was greater for lased than for unlased enamel. Qualitatively, this increased
Table 4.4 Calculated solubility of unlased (pK_{HAP}=120) and lased (pK_{HAP}=130) HAP in synthetic saliva

<table>
<thead>
<tr>
<th>Dissolution medium</th>
<th>Solubility</th>
<th>Quantity of HAc to reach K_{HAP}</th>
<th>pH at which the K_{HAP} is reached</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>HAP 4.82 x 10^{-7}</td>
<td>Lased HAP 1.83 x 10^{-7}</td>
<td></td>
</tr>
<tr>
<td>Synthetic saliva (pH=6.6)</td>
<td>HAP -1.40 x 10^{-4}</td>
<td>Lased HAP -2.33 x 10^{-4}</td>
<td>1.68 x 10^{-3} 5.49</td>
</tr>
<tr>
<td>Synthetic saliva (pH=5.5)</td>
<td>HAP -1.71 x 10^{-6}</td>
<td>Lased HAP -8.81 x 10^{-5}</td>
<td></td>
</tr>
<tr>
<td>Synthetic saliva (pH=5.0)</td>
<td>HAP 1.06 x 10^{-4}</td>
<td>Lased HAP -4.24 x 10^{-5}</td>
<td></td>
</tr>
<tr>
<td>Synthetic saliva (pH=4.5)</td>
<td>HAP 3.81 x 10^{-4}</td>
<td>Lased HAP 7.41 x 10^{-5}</td>
<td></td>
</tr>
<tr>
<td>Synthetic saliva (pH=4.0)</td>
<td>HAP 1.00 x 10^{-3}</td>
<td>Lased HAP 3.37 x 10^{-4}</td>
<td></td>
</tr>
</tbody>
</table>

1. Synthetic saliva, 1.5 mM Ca, 1.0 mM PO4, 10 mM acetate, ionic strength adjusted to 0.15 by NaCl, pH adjusted to 6.6 by adding HAc.
effectiveness of common ions as a result of lasing constitutes a synergistic relationship between the two approaches to reduction of acid susceptibility of enamel. More importantly to the mechanistic understanding of the laser effect, the common ion data unambiguously showed that the effective driving force for dissolution was reduced to about $10^{-130}$. This is the value previously reported for site #2 of the two site model for HAP dissolution (Fox et al., 1978; Higuchi et al., 1984).

One possible interpretation of the apparently very small amount of enamel converted to site #2 is that the laser irradiation is almost completely absorbed in the first micron or so of enamel and there is therefore a zone of completely converted enamel of about this magnitude. Alternatively, heat conduction could lead to a considerably broader zone of enamel being effected. The same amount of apparently less soluble mineral could represent a lessened dissolution rate for the outer unit cell of each crystallite (~5% of the crystallite mass) through about the first 10-20 microns of enamel. Such a mechanism could operate, for example, if annealing were related to loss of carbonate and this were limited to the outer unit cell simply by no convenient pathway being available for carbonate loss from the interior of a crystallite.

Laser irradiation in the range of 65 - 130 J/cm$^2$ had no measurable effect on hydroxyapatite. However, with much larger energies (1600J/cm$^2$), the hydroxyapatite IDR was reduced to the same degree as was that of enamel and carbonate apatite with 130J/cm$^2$. The effect of temperature paralleled that of laser irradiation. Again, the IDR reduction of carbonate apatite at moderate temperatures (400°C - 600°C) was consistent with a reduction in the driving force ion activity product from $10^{-120}$ to $10^{-130}$. At these temperatures there was no effect on the IDR of hydroxyapatite. At high temperatures (~1200°C), however, the IDR of hydroxyapatite was reduced by about a factor of 4 (Higuchi et al., 1986), which is consistent with the conversion of more labile site #1 of the two site dissolution model (Fox et al., 1978) to the less labile site #2. That is, the dissolution driving force IAP was reduced from $10^{-120}$ to $10^{-130}$. 
The rational design of laser irradiation treatments for increasing the resistance of enamel to dental caries requires a quantitative understanding of the interaction of laser with tooth enamel. The results reported above provide a basis for using CAP as a model system in developing this understanding since its behavior with respect to IDR reduction by laser closely parallels the behavior of human enamel. Furthermore, the parallel between the effects of heat treatment support the hypothesis that the CO₂ laser acts via its thermal effect.

CAP has previously been suggested as an impurity ion that perturbs the behavior of the system. This study is the most dramatic demonstration to date of this idea. Indeed, with respect to the laser effect, carbonate could be regarded as much more than an impurity ion; the presence of 2% carbonate has essentially transformed hydroxyapatite into a qualitatively different mineral phase.

Conclusions

Experimental results in our laboratories strongly support the hypothesis that laser irradiation exerts its effect by lowering the apparent solubility of enamel. There are several parts to the rationale for this conclusion, which will be discussed in turn below:

1. Previous investigations suggested that heat treatment or laser treatment of synthetic HAP (0% carbonate) at high temperatures (1200°C) lowers its apparent solubility. The apparent solubility product (Kₜₜₜ) changes by 10 orders of magnitude, from about 10⁻¹²₀ to 10⁻¹³₀.

2. This chapter suggested that heat treatment or laser treatment of synthetic C-HAP (1.9% carbonate) showed a similar solubility reduction in a much lower (400-500°C) temperature range.

3. Laser treatment can be used to achieve the appropriate temperature (400-500°C) in enamel and therefore achieve solubility reductions similar to those resulting from high temperature heat treatment (1200°C) of HAP.
4. Carbonated apatite is a better model for studying the effect of laser irradiation on human dental enamel.

5. Laser treatment reduces the apparent solubility of human enamel from that described by an ion activity product (based on \((\text{Ca})_{10}(\text{PO}_4)_6(\text{OH})_2)\) of about \(10^{-120}\) to about \(10^{-130}\). The 10 orders of magnitude difference in the solubility product corresponds to about a factor of 4 to 5 difference in molar solubility. Model calculations show that the threshold pH for the onset of demineralization of enamel by acids is lowered by about 0.7 pH unit by the above change in apparent solubility.
CHAPTER 5

PHYSICAL APPROACH TO IMPROVE
CO₂ LASER EFFECT

Introduction

The general objective of this thesis is not only to show that laser treatment can inhibit caries, but also to show how to improve the treatment to achieve maximum caries inhibition. This objective can be achieved by a physical (engineering) approach and a chemical (pharmaceutical) approach. This chapter will emphasize the physical approach. The chemical approach will be discussed in Chapter 6.

The working hypotheses for the physical approach are centered around the idea that the key parameter in determining the "effectiveness" of laser irradiation is the "zone" of enamel that is made more acid resistant by the laser treatment. Therefore, the purpose of work presented in this chapter was to try to control the resistance of the "zone" by controlling the lasing conditions.

We shall assume that the laser effect is completely due to the heat effect demonstrated in Chapter 4. The temperature profile developed in the enamel as a result of laser irradiation can therefore be directly related to the resistance of the "zone." If that is the case, then, the lasing parameters controlling the thickness of the "zone" will be the same as those lasing parameters controlling the temperature profile.

From an engineering point of view, laser irradiation of human enamel is a standard heat transfer problem. There are three heat transfer processes coexisting during the laser irradiation process, i.e., conduction, convection, and radiation. These heat transfer processes (especially conduction and radiation) can be controlled by varying laser operating
parameters, including: the total amount of energy, the wavelength at which it is delivered and the rate at which it is delivered. Therefore, the temperature gradient developed in the enamel as a result of laser irradiation can be controlled in a predictable way by varying those laser operating parameters.

Featherstone and Nelson have specifically investigated the effects of various wavelengths of CO₂ laser irradiation on subsurface demineralization (Nelson et al., 1987; Featherstone et al., 1987). Their findings are particularly important since they showed the caries inhibition effect by CO₂ laser is wavelength dependent. However, no systematic research has been done on the caries inhibition effect by varying other operating parameters.

Therefore, the components of the physical approach described in this chapter are as follows:

1. Calculate the time and position dependent temperature profile of the enamel - which can be done by developing an appropriate mathematical model for computer simulation of the temperature profile.
2. Measure the resistance of the "zone" - which can be directly related to the resistance of the enamel to acid challenge as measured by X-ray microradiography.
3. Test the hypothesis that the "effectiveness" of the laser treatment can be related to the time/position dependent temperature profiles resulting from different energy densities and pulse patterns.

Materials and methods

X-ray determination of mineral density profiles

The enamel sample after dissolution was embedded in poly-methyl-methacrylate (PMMA) for 24 hr and then fixed at 60°C for 12 hr before sectioning. Sections approximately 200 microns thick were cut through the exposed window perpendicular to the enamel surface. The section to be examined was then ground by using 600 grit silicon
carbide paper to a thickness of exactly 100 microns. The thin section was placed in direct contact with the emulsion on a 2"x2" high resolution glass photographic plate (Type I-A, Eastman Kodak Co, Rochester, NY). A wedge of bovine enamel was placed adjacent to the surface to be analyzed. This untreated enamel wedge is used as an internal standard to factor out any artifacts resulting from deviations from linearity in the time/temperature dependent film development process.

The sample was placed in the center of an X-ray beam (Faxitron, Hewlett-Packard, McMinnville, OR) for 25 min. The negative was developed in D-19 developer (Eastman Kodak Co., Rochester, NY).

Density profiles were quantified from the microradiographic images by using a Leitz microscope photometer equipped with a scanning stage. The sample image was analyzed by moving it in 0.25 micron steps and at each step measuring the light transmitted through an aperture 0.25 micron wide. The stage movement was controlled by a microcomputer which also recorded the transmitted light by means of an analog to digital converter connected to the photomultiplier head of the microscope. The range of stage travel was 100 microns so that each image consisted of some 400 separate readings, each of which was an average of one to eight individual readings as selected. The image of the enamel wedge internal standard was recorded in a similar fashion.

The raw data from the image scan was processed in the following way. A calibration curve (ADC reading versus enamel thickness) was constructed for the enamel wedge by fitting a fourth degree polynomial to the logarithm of ADC reading versus enamel thickness. This procedure also included an algorithm that precisely locates the edge of the wedge based on an examination of the data. This calibration curve for the wedge was then used to convert the ADC versus position data for the sample to equivalent enamel thickness (or percentage of original mineral remaining) versus thickness. The data at each stage of this process (sample raw data, wedge raw data and calibration curve, sample density versus position) were then plotted as desired.
The mathematical model

A diagrammatic representation of structural components of the human enamel block is shown in Figure 1.3. The complexities of the tooth geometry require significant simplifications in the mathematical model. The enamel block was modeled as a cylinder with a height $L$ and a radius $r$ as shown in Figure 5.1. This cylinder is assumed to be axisymmetric and homogeneous with constant physical properties. The standard three dimensional heat conduction equation

$$\frac{\partial T}{\partial t} = \nabla \cdot \alpha(T) \nabla T$$

where

- $T$ = temperature change ($^\circ$C),
- $t$ = time (sec),
- $\alpha$ = thermal diffusivity (cm$^2$/sec),

which is the combination of Fourier's conduction law and the law of conservation of thermal energy, is the backbone for our mathematical analysis. The temperature $T$ here was defined as the temperature difference rather than absolute temperature so that we don't have to consider the ambient temperature in our temperature calculation. The thermal diffusivity was assumed to be a constant in our enamel model and Equation 5.1 can be simplified as

$$\frac{\partial T}{\partial t} = \alpha \nabla^2 T.$$  \hspace{1cm} (5.2)

Equation 5.2 has been applied by most other investigators for study theoretical temperature profiles for laser irradiated human tooth since 1974 (Boehm et al., 1975; Laufer and Haber, 1985; Sagi, 1990). A cylindrical coordinate system was chosen for all the previous analyses. Equation 5.2 can be simplified to the following two-dimensional equation
\[
\frac{\partial T}{\partial t} = \alpha \frac{\partial^2 T}{\partial x^2} + \alpha \frac{1}{\rho c} \left( \frac{\partial T}{\partial t} \right)
\]  
\hspace{1cm} (5.3)

If the lasing beam size is large enough to cover the whole enamel surface as shown in Figure 2, the laser energy input can be assumed to be uniformly distributed on one of the end surfaces of the cylinder and Equation 5.3 can be further simplified to a one-dimensional problem as follows:

\[
\frac{\partial T}{\partial t} = \alpha \frac{\partial^2 T}{\partial x^2} 
\]  
\hspace{1cm} (5.4)

Laser energy is absorbed from the lasing surface boundary. At both boundaries of the slab, heat loss also occurs as a result of convection, resulting in the following boundary conditions:

at \( x = 0 \) \[ \frac{\partial T}{\partial x} = \frac{h_1}{k} T + \frac{Q}{k} \]

at \( x = L \) \[ \frac{\partial T}{\partial x} = -\frac{h_2}{k} T \]

where

\[
Q = \text{laser energy input function (cal/sec/cm}^2) \\
h_1 = \text{heat transfer coefficients at lasing surface (cal/cm}^2 \degree C \text{sec)} \\
h_2 = \text{heat transfer coefficients at the other side (cal/cm}^2 \degree C \text{sec)}
\]

Both equation 5.3 and 5.4 have been solved numerically (Boehm et al., 1975; Laufer and Haber, 1985). Common problems associated with all the previous approaches are

1. The laser energy input function was introduced via the boundary conditions. In this case the absorption coefficient can not be considered and such a model can
only be applied to a laser beam which is highly absorbed by the target enamel (e.g. CO₂ laser)

2. Since the numerical algorithm was not able to consider the heat loss from both surfaces, the insulated boundary assumption was generally applied.

3. The finite difference and finite element algorithms used were very time-consuming and are not well suited for calculating multiple dosage and very short pulse laser irradiation patterns.

In order to take those factors into consideration, equation 5.4 has been modified by us to account for absorption of energy according to Beer's law as follows:

\[ \frac{\partial T}{\partial t} = \alpha \frac{\partial^2 T}{\partial x^2} + \beta \cdot M(\lambda) \cdot Q \exp (-M(\lambda)x) \quad (5.5) \]

where

- \( \alpha = \) thermal diffusivity (cm²/sec)
- \( \beta = \frac{1}{\rho C_p} \)
- \( \rho = \) density (g/cm³)
- \( C_p = \) specific heat (cal/g°C)
- \( k = \) thermal conductivity (cal/sec/cm°C)
- \( M(\lambda) = \) absorption coefficient, extinction coefficient (cm⁻¹)

boundary conditions were also simplified as follows:

\[
\begin{align*}
\text{at } x = 0 & & \frac{\partial T}{\partial x} = \frac{h_1}{k} T \\
\text{at } x = L & & \frac{\partial T}{\partial x} = -\frac{h_2}{k} T
\end{align*}
\]
Equation 5.5 is unique from all previous investigators in that the lasing energy was introduced as a heat source in the differential equation and not via the boundary conditions. This allows wavelength dependent absorption of laser energy to be considered. Also, since the boundary conditions were simplified, the heat transfer coefficient can be easily applied to this model.

**Laplace transform technique for solving the thermal diffusion equation**

Equation 5.5 is a parabolic type partial differential equation. The complexity of both the heat generation term and boundary conditions makes it difficult to solve analytically (Carslaw, 1954; Crank, 1975). This equation is also difficult to solve numerically due to the "stiff" (Gear, 1971) characteristics of the model itself especially with very high absorption coefficient. Traditional finite difference algorithms can not efficiently solve this problem because those methods are not sufficiently stable for practical integrator step sizes (Burden et al., 1981). The newly developed LSODE/DSS2 stiff parabolic partial differential equation code with adjustable step size (Morris et al., 1968; Schiesser et al., 1982) also failed to solve this equation.

The approach taken in this study for successfully solving equation 5.5 was "Laplace transforms." Essentially, the Laplace transform is a technique whereby a differential equation is converted into an equivalent algebraic form which can be solved by the laws of elementary algebra. There are many such operational methods but the Laplace transformation is particularly useful for this heat transfer problem because the boundary conditions are introduced into the equation prior to its solution. The first step of applying the Laplace transform technique to solve equation 5.5 is converting it into an ordinary differential equation which can be readily solved. The procedure for that have been well established (Carslaw, 1954) and will not be discussed in detail here. The equivalent algebraic solution of equation 5.5 after Laplace transformation can be expressed as
\[ \Theta = \frac{\alpha \beta M(\lambda)Q_0}{\alpha M(\lambda)^2 - S} \left[ \frac{\text{term 1} + \text{term 2}}{\text{denom}} - \frac{\exp (-M(\lambda)L)}{\alpha} \right] \]  

(5.6)

where

\[ S = \text{time constant (sec}^{-1}) \]

\[ \Theta = \text{Laplace transformed temperature (°C)} \]

\[ \text{term 1} = \left( M(\lambda) + \frac{h_1}{k} \right) \left( q \cosh (q[L - x]) + \frac{h_2}{k} \sinh (q[L - x]) \right) \]

\[ \text{term 2} = \left( M(\lambda) + \frac{h_2}{k} \right) \left( q \cosh (qx) + \frac{h_1}{k} \sinh (qx) \right) \exp (-M(\lambda)L) \]

\[ \text{denom} = \left[ \left( S + \alpha \frac{h_1 h_2}{k^2} \right) \sinh (qL) + \alpha \frac{q}{k} (h_1 + h_2) \cosh (qL) \right] \]

\[ q = \sqrt{\frac{S}{\alpha}} \]

The second step of Laplace transform technique is called "inversion". The transformed temperature (\( \Theta \)) is inverted back to normal temperature (\( T \)) numerically using a hybrid algorithm incorporating Piessens' method and/or Weeks' method (Piessen et al., 1984; Weeks et al., 1986) as dictated by problem conditions. This transform inversion program was kindly provided by MicroMath Scientific Software, Salt Lake City, Utah.

The detailed method for carrying out such an inversion won't be discussed here. The Laplace transform technique can not only overcome all those difficulties mentioned previously, but also readily handles with the pulsed laser and multiple lasing dosage case.
Results and discussion

Mineral density profile assessment of the effect of lasing energy on the acid resistant "zone" during prolonged exposure of enamel to acid

Human enamel slabs were irradiated at four combinations of time and laser intensity as follows:

Case 1. 25W for 4 sec, 65 J/cm²
Case 2. 25W for 8 sec, 130 J/cm²
Case 3. 50W for 2 sec, 65 J/cm²
Case 4. 50W for 4 sec, 130 J/cm²

and then exposed to a solution with a hydroxyapatite ion activity product of 10⁻¹²⁶. The purpose of this was twofold. First, we expected no dissolution from annealed crystals (pK_{HAP} < 10⁻¹²⁶) so that the zone thickness could be detected. Second, this solution approximates the composition of the saliva during a caries challenge. Because of the difficulty of accurately measuring dissolution rates in the presence of background calcium and phosphate, we have evaluated these experiments by measuring mineral density profiles after 24 hr exposure to the dissolution medium as shown in Figures 5.2 and 5.3.

As can readily be seen, all the lased samples showed less dissolution than the control samples. There was some subsurface dissolution after 24 hr exposure for the samples receiving the higher total energy (130 J/cm²) exposure, while there was surface recession for the control samples and the samples receiving the lower total energy (65 J/cm²) exposure. This kind of subsurface dissolution phenomena indicates the surface zone of enamel has a higher solubility (pK_{HAP} > 126) than the inside. This is consistent with the conclusion in the previous chapter that "laser treatment reduces the apparent solubility of human enamel from that described by an ion activity product (based on (Ca)₁₀(PO₄)₆(OH)₂) of about 10⁻¹²⁰ to about 10⁻¹³⁰."
Figure 5.2 Mineral density profiles of lased human enamel (25W) after dissolution in partial saturated buffer solution, pH_{\text{HAP}}=126, for 24 hr (pH=4.5, \mu=0.5, 0.1M acetate buffer)
Figure 5.3  Mineral density profiles of lased human enamel (50W) after dissolution in partial saturated buffer solution, pK_{HAP}=126, for 24 hr (pH=4.5, μ=0.5, 0.1M acetate buffer)
The thickness of the surface zone can also be estimated from Figures 5.2 and 5.3 and compared with the theoretical temperature profiles calculated later. The 24-hr experiments also showed that the rate at which the energy was applied (130J/cm² delivered within 4 or 8 sec) also has an effect on the response to acid challenge. The level of protection achieved in these experiments would be expected to be adequate against the most severe acid challenges that might be encountered in vivo.

**Effect of laser energy on temperature profiles developed during laser irradiation**

For purposes of calculating the temperature profile, a value for each parameter in equation 5.5 had to be assigned. All the quantities except the absorption coefficient (extinction coefficient) in the equation have been reported as shown in Table 5.1 (Brown et al., 1970). The only adjustable parameter when applying equation 5.5 to different types of laser is the absorption coefficient. The absorption coefficient can be calculated by transmission experiments along with appropriate photon distribution model (Welch, 1987). The model applied in the current study is the simplest Beer's law model. The extinction coefficient for CO₂ laser (λ=10.6 μm) can be derived from the IR spectra assuming that Beer's law is obeyed. An absorption coefficient value of 1000 cm⁻¹ was used for CO₂ laser throughout this study. This number is consistent with the finding reported by other researches which indicates the penetration depth into enamel by CO₂ laser (10.6 μm) is only a few μm (Muller, 1990).

Computer simulated time-dependent surface temperatures for each set of lasing conditions are shown in Figures 5.4-5.7. Two important conclusions can be drawn from these data:

1. For the same power laser irradiation cases (Case1 and Case 3, Case 2 and Case 4), surface temperature is proportional to total energy density.
Table 5.1 Thermo-physical properties used for equation 5.5

<table>
<thead>
<tr>
<th>Properties</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\rho$ (density)</td>
<td>2.8 g/cm$^3$</td>
</tr>
<tr>
<td>$C_p$ (specific heat)</td>
<td>0.17 cal/g$^\circ$C</td>
</tr>
<tr>
<td>$k$ (thermal conductivity)</td>
<td>0.00223 cal/sec/cm$^2$$^\circ$C</td>
</tr>
<tr>
<td>$\alpha$ (thermal diffusivity)</td>
<td>0.00409 cm$^2$/sec</td>
</tr>
<tr>
<td>$\beta$ (1/$\rho C_p$)</td>
<td>2.101 cal/cm$^3$$^\circ$C</td>
</tr>
<tr>
<td>$M(\lambda)$ (absorption coefficient)</td>
<td>1000 cm$^{-1}$</td>
</tr>
<tr>
<td>$h_1, h_2$ (heat transfer coefficients)</td>
<td>0 cal/cm$^2$$^\circ$C/sec</td>
</tr>
<tr>
<td>$L$ (thickness of enamel block)</td>
<td>5.0* mm</td>
</tr>
<tr>
<td>$r$ (diameter of enamel surface)</td>
<td>1.0* cm</td>
</tr>
<tr>
<td>$R$ (diameter of laser beam)</td>
<td>1.4* cm</td>
</tr>
</tbody>
</table>

* those are hypothetical values for calculating the temperature but they are close to the real experimental conditions used by Fox et al. (1991).
Figure 5.4  Surface temperature profile for lased enamel (25W, 4sec=65J/cm²)
Figure 5.5  Surface temperature profile for lased enamel (50W, 2sec=65J/cm²)
Figure 5.6  Surface temperature profile for lased enamel (25W, 8sec=130J/cm²)
Figure 5.7 Surface temperature profile for lased enamel (50W, 4sec=130J/cm²)
2. For the same total energy laser irradiation cases (Case 1 and Case 2, Case 3 and Case 4), surface temperature is proportional to the laser power applied.

Although the calculated temperature profiles do not match exactly the experimentally measured temperature (Chapter 7), the above conclusions were still valid since they were based on relative comparisons.

The position-dependent temperature profiles at the end of lasing period are also shown in Figures 5.8-5.11. As can be seen, the maximum temperature extends from surface inward, forming a high temperature zone. Different depths of this zone thickness can be achieved by using different lasing conditions as shown in Figures 5.8-5.11. However, most importantly, as mentioned earlier, different temperatures were achieved by different energy delivery patterns (power densities) even though the total energies were the same. This finding is consistent with the mineral density profile data, where different mineral densities resulted from different energy delivery patterns even though the total energies were the same. The remaining mineral amount in the surface zone was the basis for qualitative comparison.

Optimization of irradiation conditions by varying wavelength and pulse duration

The best caries inhibition situation was achieved by high energy and power density (Case 4) as shown in Figure 5.3. As can be seen from the mineral density profile, there are still about 20 microns of erosion from the enamel surface. This 20 microns erosion implies that the irradiation conditions investigated in this chapter are not optimal. The energy level can't be further increased since 130J/cm² laser irradiation has already led to browning of teeth which would not be clinically acceptable. One way to overcome this is by the chemical approach which will be discussed in the next chapter. Another method is to change the laser conditions investigated in this chapter.
Figure 5.8 Maximum temperature profile for lased enamel (25W, 4sec=65J/cm²)
Figure 5.9  Maximum temperature profile for lased enamel (50W, 2sec=65J/cm²)
Figure 5.10  Maximum temperature profile for lased enamel (25W, 8sec=130J/cm²)
Figure 5.11 Maximum temperature profile for lasered enamel (50W, 4sec=130J/cm²)
In order to further demonstrate the influence of pulse duration, computer simulated position dependent temperature profiles were done for one specific energy density (65J/cm²) but for different pulse durations as shown in Figures 5.12-5.16. The temperature variation can be clearly seen. One important conclusion from those calculations is that the maximum temperature achieved in the enamel is inversely proportional to the pulse duration.

Let us assume that 400°C is the necessary temperature for annealing enamel and that an annealing zone of 10-20 microns or less will avoid pulp damage. As shown by Figure 5.17, a long duration of lasing heats a much larger zone of enamel (several hundred microns) than necessary in order to achieve high enough temperatures in the first 10-20 microns. Figure 5.18 shows that a reduction of the duration of irradiation to 10 msec would allow heating of the first 20-50 microns to 400°C with about a 10-fold decrease in applied energy.

The importance of wavelength is also considered here. In case of the submillisecond pulses, only a few microns will be heated at the 10.6µ (M = 1000 cm⁻¹) wavelength as shown in Figure 5.19. But by shifting to a less efficiently absorbed wavelength (e.g. 13µ, M = 100 cm⁻¹), the same submillisecond pulse could heat about the first 20 microns of enamel as shown in Figure 5.20.

**Conclusions**

A qualitative correlation between laser induced acid resistance and the calculated temperature profiles developed in the enamel has been achieved. It is conceivable that the "effectiveness" of laser irradiation can be optimized by controlling the lasing parameters to maximize the extent of formation of less soluble mineral, while avoiding thermal damage to the pulp. The conclusions from this chapter are:

1. Theoretical temperature profiles during laser irradiation of enamel were successfully simulated by developing a physical model and then solved by a
Figure 5.12  Maximum temperature profile for lased enamel (50W, 2sec=65J/cm²)
Max Temp (65J/cm², 1sec)

Figure 5.13  Maximum temperature profile for lased enamel (100W, 1sec=65J/cm²)
Figure 5.14 Maximum temperature profile for lased enamel ($10^3 W, 10^{-1} \text{sec} = 65 \text{J/cm}^2$)
Figure 5.15  Maximum temperature profile for lased enamel (10^4 W, 10^{-2} sec=65 J/cm^2)
Max Temp (65J/cm², 0.001sec)

Figure 5.16  Maximum temperature profile for lased enamel (10⁵W, 10⁻³sec=65J/cm²)
Figure 5.17  Maximum temperature profile for lased enamel (65J/cm², 2sec)
Figure 5.18 Maximum temperature profile for lased enamel (5.85J/cm², 0.01sec)
Figure 5.19 Maximum temperature profile for lased enamel (9.75 J/cm^2, 0.1 msec)
Figure 5.20  Maximum temperature profile for lased enamel (1.3J/cm², 0.1msec)
"Laplace Transform" algorithm. This model differs from previous investigations in that both the laser wavelength and the environmental heat transfer coefficient were included. The development of such a laser heat transfer model is one particular contribution of this thesis work. This model can be applied to laser applications other than caries prevention (e.g. surgery and military) within the assumptions of the model. One important limitation of this model is that all the physical parameters (thermal diffusivity and thermal conductivity) are temperature independent. The importance of this assumption will be further assessed in Chapter 7.

2. The subsurface dissolution behavior seen from mineral density profiles is further evidence that enamel surface crystal has been annealed to site #2 (pK_{HAP}=130) as concluded from Chapter 4.

3. The temperature profiles developed in the enamel can be related to the acid resistance of human teeth after laser irradiation by comparing the theoretical temperature profiles with experimental mineral density profiles.

4. Higher total energy density (same power density) gave stronger acid resistance. Shorter pulse duration (same energy density) gave stronger acid resistance. This is consistent with the calculated temperature profile where higher total energy density (same power density) gave higher temperature and shorter pulse duration (same energy density) gave higher temperature.

5. Continuous wave irradiation at low power is inefficient. Shortening the total time for delivery of energy would be expected to allow the target zone to be annealed with a lower total energy expenditure. Calculations suggested that the maximal acid resistance will be attained with the energy delivered as a single pulse.

6. It has been suggested that practical clinical applicability of laser irradiation requires that a laser irradiation regimen be designed to impart this dissolution resistance to the first 10 to 20 microns of enamel mineral, without damaging other tissue.
Preliminary experiments and model calculations indicate that the achievement of such a clinically effective treatment should be feasible by pulsed laser.
CHAPTER 6

CHEMICAL APPROACH TO ENHANCE

CO₂ LASER EFFECT

Introduction

One objective of this thesis is to improve the "effectiveness" of the CO₂ laser in increasing resistance to dental caries. In addition to the physical approaches discussed in the previous chapter, chemical approaches have also been investigated. The purpose of this investigation is to see whether the combinations of laser irradiation and chemical agents is better than either alone.

The chemicals we choose were agents shown to be "inhibitors" of dental enamel dissolution (i.e., ethane-1-hydroxy-1,1-diphosphonic acid (EHDP), dodecylamine HCl (DAC), and fluoride (F)). Previous work in our laboratory shows that these agents achieve their effects by entirely different mechanisms (Yamamoto et al., 1986, Otsuka et al., 1987; Otsuka et al., 1989), although they share a common trait of being much more effective on site #2 of HAP than on site #1.

When HAP (Ca₁₀(PO₄)₂(OH)₂) is exposed to a low fluoride concentration solution, rapid fluoride for hydroxyl ion exchange occurs and a fluorapatite (FAP, Ca₁₀(PO₄)(F)₂) complex is formed on the surface, the fluoride ion (F⁻) in solution then acts via a common ion effect on the fluorapatite surface complex that controls dissolution (Mir et al., 1969; Ludwig et al., 1982; Crommelin et al., 1983, Cho et al., 1983; Wong et al., 1990). Laser irradiation reduces the effective solubility of the enamel, presumably by annealing the more labile dissolution site (site #1) as concluded from Chapter 4. This common ion effect is made more pronounced by the lowering of the driving force ion activity product (K_{FAP}).
EHDP and DAC (structures shown in Figures 6.1) have been found to completely inhibit the dissolution of heat treated synthetic HAP at concentrations of 3 mM (Otsuka et al., 1987; Otsuka et al., 1989), while dissolution of non-heat treated HAP is little affected by 3 mM levels of either DAC or EHDP. It has also been found that EHDP can totally inhibit the dissolution of laser treated (50W for 400 s, 13,000J/cm²) synthetic HAP (Higuchi et al., 1989). We attributed these results to annealing away of the more labile site #1 and the greater effectiveness of the agents on the remaining site #2 as mentioned above. This immediately leads to the hypothesis that these agents might act synergistically with laser irradiation, since the laser can presumably convert site #1 to site #2. This synergism would not only be of practical importance, but would also provide further evidence that this conversion to site #2 is the mechanism of action of the laser.

The caries inhibition effect can be maximized by controlling temperature as concluded in Chapter 5. The best caries inhibition situation was achieved by high energy and power density as shown in Figure 5.3. As can been seen from the mineral density profile, there are still about 20 microns of erosion from the enamel surface. It is expected that the chemical approach proposed in this chapter can improve this.

In this chapter we test the following two hypotheses:

1. The effect of laser irradiation can be further enhanced by those chemical dissolution rate inhibitors previously investigated in our laboratory (EHDP, DAC); the enhancement of the laser effect by these inhibitors is not only additive but can be synergistic (consistent with site #2 HAP).

2. The effect of laser irradiation can be enhanced by fluoride because laser irradiation converts the rate governing dissolution surface complex in the presence of F from the site #1 FAP (pK_FAP=120) crystal to site #2 FAP (pK_FAP=130).
Figure 6.1 Chemical structures for disodium ethane-1-hydroxy-1,1-diphosphonic acid (EHDP) and dodecylamine hydrochloride (DAC)
Materials and methods

Dodecylamine hydrochloride (DAC)

Reagent grade DAC (Eastman Kodak Co.) was recrystallized once in benzene. The purity was verified by means of paper chromatography.

Ethane-1-hydroxy-1,1-diphosphonic acid (EHDP)

The EHDP was kindly provided by Norwich Eaton Pharmaceutical, Inc. The purity of the EHDP was 99.8%.

Fluoride (F)

Fluoride was added from a 100 ppm stock solution in the form of sodium fluoride (NaF).

Solutions

Acetate buffer solutions (0.10M, pH=4.5, ionic strength=0.5M) were prepared by mixing calculated quantities of acetic acid, sodium acetate and sodium chloride along with calculated amounts of NaF, DAC, or EHDP and using HCl or NaOH if necessary to adjust the pH as determined by a digital pH meter (Altex Model 4500). Required amounts of the acid and its salt and the sodium chloride background electrolyte were calculated with a commercially available computer program (EQUIL, MicroMath Scientific Software, Salt Lake City, UT). The measured pH was generally within ±0.01 unit of the calculated pH.

Design of solution compositions for common ion studies

The expected suppression of initial dissolution rates by various common ion concentrations was calculated as follows. It was assumed that dissolution rate would be proportional to an apparent solubility, governed by an ion activity product (IAP) of the form (Ca)\(_{10}\) (PO\(_4\))\(_6\) (F)\(_2\) while the crystal structure is C-HAP, (Na)\(0.305\)(Ca)\(9.695\)(PO\(_4\))\(5.695\)(CO\(_3\))\(0.305\)(OH)\(_2\) (Wong et al., 1990). For various assumed values of this IAP ranging from \(10^{-115}\) to \(10^{-133}\), the ratios of dissolution rate in the
presence of a specified common ion concentration to that for no common ion were calculated. Calculations were carried out by a commercially available computer program, EQUIL (MicroMath Scientific Software, Salt Lake City, UT 84121). All calculations assumed the presence of 0.10M acetate buffer at pH=4.5 with ionic strength adjusted to 0.5M by addition of NaCl. Based on these calculations and the hypothesis that lasing would change the driving force from an IAP of $10^{-120}$ to about $10^{-130}$, calcium concentrations of 0.7 and 1 mM and phosphate concentrations of 1.5 and 2.5 mM were chosen for the common ion studies. The expected dependencies for dissolution rate on calcium and phosphate common ion concentrations are similar to those shown in Table 4.1. As the table shows, these concentrations should allow us to experimentally differentiate the $pK_{\text{HAP}}=130$ case from the $pK_{\text{HAP}}=120$ case, and also to differentiate both from the control case (0.1 ppm and 0.5 ppm fluoride).

**Dissolution rate and mineral density study**

Two experimental methods (i.e. mineral density and initial dissolution rate) were used in this study to assess the effect of chemical inhibitors. Both methods have been discussed in previous chapters.

**Results**

**Effects of lasing and EHDP and DAC on initial dissolution rate**

Figure 6.2 shows the dissolution of lased (50W for 2 sec = 65J/cm²) and unlased enamel in sink buffer (0.1M acetate buffer, pH=4.5) and in a similar buffer containing 3 mM EHDP. Figure 6.3 shows the dissolution of lased (50W for 2 sec, 65 J/cm²) and unlased enamel in sink buffer (0.10M acetate buffer, pH=4.5) and in a similar buffer containing 3 mM DAC. In each case there is no detectable dissolution of the lased sample after 5 min exposure to sink buffer containing the chemical agent, even though the control had lost about 1 micron of enamel in this time. After 15 min, however, there
Figure 6.2 Dissolution of lased (65J/cm²) and unlased human enamel in sink solution

- (□) unlased enamel (no EHDP);   (○) lased enamel (no EHDP)
- (■) unlased enamel (3mM EHDP);   (●) lased enamel (3mM EHDP)
Figure 6.3 Dissolution of lased (65J/cm²) and unlased human enamel in sink solution

- □ unlased enamel (no DAC)
- ○ lased enamel (no DAC)
- ■ unlased enamel (3mM DAC)
- ● lased enamel (3mM DAC)
has been dissolution in each case, although at much lower levels than observed for the control or for either laser treatment or chemical treatment alone. The initial dissolution rate data shows only that the effect of lasing (65J/cm²) can be enhanced by chemical inhibitors, but does not convincingly show synergism.

**Synergistic effects of lasing and EHDP or DAC in dissolution reduction during prolonged exposure of enamel to acid**

Since the initial dissolution rate studies showed detectable dissolution after 15 min exposure to the acetate buffer solution, studies were carried out for samples that had received twice the exposure of laser irradiation (50W for 4 sec, 130 J/cm²). A much more severe dissolution test was then conducted. Samples were exposed to dissolution media for 24 hr and the resulting mineral loss measured by quantitative microradiography. As shown in Figure 6.4, laser treatment alone was only somewhat effective in improving the resistance of enamel to this very strong acid and prolonged challenge. The severity of the challenge is evident from the approximately 150 microns of enamel lost when unlased teeth were exposed to acid buffer without either of these chemical agents. The higher irradiation level of 130 J/cm² was about 30% more effective in reducing mineral loss than was the lower 65 J/cm² exposure.

The combination of 130 J/cm² and these chemical agents was much more dramatic. Figure 6.5 shows the mineral density profiles resulting from acid challenge in the presence of 3 mM EHDP for both lased and unlased enamel blocks. As can be seen, the increase from 65 to 130 J/cm² decreases the enamel loss from about 25 microns to almost zero. The results for 3 mM DAC shown in Figure 6.6 are similar, although in each case DAC was more effective than EHDP in its corresponding experiment. Again, the combination of the higher laser irradiation and the chemical agent afforded virtually complete protection from a severe acid challenge for 24 hr.
Figure 6.4  Mineral density profiles of lased and unlassed human enamel under sink conditions (pH=4.5, μ=0.5, 0.1M acetate buffer)
Figure 6.5 Mineral density profiles of lased and unlased human enamel under sink conditions (pH=4.5, μ=0.5, 0.1M acetate buffer, 3mM EHDP)
Figure 6.6 Mineral density profiles of lased and unlased human enamel under sink conditions (pH=4.5, μ=0.5, 0.1M acetate buffer, 3mM DAC)
The synergism effect was best demonstrated by comparing Figures 6.5 and 6.6 with Figure 6.4. Lasing alone (130J/cm²) inhibits dissolution from about 150 microns to 45 microns (~75% reduction), and EHDP alone inhibited dissolution from about 150 microns to 50 microns (~66% reduction). The combined effect should inhibit dissolution from 150 to 15 microns (75% + 66% x 25% = 91% or 66% + 75% x 34% = 91%) if this effect is simply additive. As can be seen clearly from Figure 6.5, zero dissolution (100% reduction) was achieved by the combined treatment. However, for the low energy lasing cases (65J/cm²), a simple additive effect was observed and can be seen in Figures 6.5 and 6.6.

Effects of lasing and F on enamel dissolution

The dissolution suppressing effect of fluoride was also enhanced by pretreatment with laser irradiation. Figure 6.7 shows a typical dissolution profile of lased (50W for 2 sec = 65J/cm²) and unlased enamel in sink buffer (0.1M acetate buffer, pH=4.5) and in a similar buffer containing 0.5ppm fluoride. Figure 6.8 shows the mineral density profiles for enamel samples exposed to an acid challenge with 1 ppm fluoride in the solution. As can be seen from both figures, the effect of laser on reducing the initial dissolution rate was enhanced by the fluoride. The conditions used in this experiment differ from those that might be experienced in the clinical situation in that the fluoride level in the experiment is somewhat higher, and there is no calcium or phosphate common ion present. We chose the no common ion condition to provide the maximum opportunity for dissolution to occur.

Dissolution rate driving force in the presence of fluoride

The dissolution driving force is assumed to be governed by (Ca)_{10}(PO_{4})_{6}(F)_{2} surface complex when fluoride is present (Wong et al., 1990). For this study, the suppression of IDR (initial dissolution rate) by various concentrations of calcium or phosphate common ion was measured as shown in Table 6.1. As was the case in Chapter 4 when no fluoride was present, the pK_{FAP} values for lased enamel were statistically similar for all solution
Figure 6.7 Dissolution of lased (65J/cm²) and unlased human enamel in sink solution
(□) unlased enamel (no F);  (O) lased enamel (F)
(■) unlased enamel (0.5ppm F);  (●) lased enamel (0.5ppm F)
Figure 6.8 Mineral density profiles of lased and unlased human enamel under sink conditions (pH=4.5, μ=0.5, 0.1M acetate buffer, 1ppm F)
Table 6.1 Initial dissolution rate results for common ion experiments

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Common ion</th>
<th>IDR(^1) control IDR</th>
<th>pK(_{FAP})</th>
<th>best fit(^2) pK(_{FAP})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lased</td>
<td>0.1 ppm F</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(50W, 2sec)</td>
<td>0.1 ppm F</td>
<td>69.8±2.0%</td>
<td>128.9 - 130.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.7 mM Ca</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1 ppm F</td>
<td>44.5±1.9%</td>
<td>127.5 - 129.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5 mM PO4</td>
<td></td>
<td></td>
<td>129.1±0.2 (128.4-129.8)</td>
</tr>
<tr>
<td></td>
<td>0.5 ppm F</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5 ppm F</td>
<td>54.1±2.8%</td>
<td>128.1 - 130.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 mM Ca</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5 ppm F</td>
<td>54.9±1.7%</td>
<td>127.9 - 129.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.5 mM PO4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unlased</td>
<td>0.1 ppm F</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1 ppm F</td>
<td>91.9±3.3%</td>
<td>116.4 - 122.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.7 mM Ca</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1 ppm F</td>
<td>75.0±8.1%</td>
<td>116.2 - 122.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5 mM PO4</td>
<td></td>
<td></td>
<td>119.3±0.4 (118.2-120.5)</td>
</tr>
<tr>
<td></td>
<td>0.5 ppm F</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5 ppm F</td>
<td>86.8±3.2%</td>
<td>117.1 - 120.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 mM Ca</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5 ppm F</td>
<td>84.1±5.3%</td>
<td>115.5 - 121.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.5 mM PO4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. IDR ratio (%) was determined by average of three experimental runs.
2. best fit pK\(_{FAP}\) was calculated by all the IDR ratio (%), the 95% confidence range was shown in parenthesis.
conditions as were the values for the dissolution experiments with unlaesed enamel.

Best fit values for pK_FAP were determined similarly to the best fit pK_HAP values in Chapter 4 and are summarized also in Table 6.1. Again lasing had the anticipated effect of reducing the apparent solubility of enamel from pK_FAP=118 to pK_FAP=130. As was the case in the absence of fluoride (Chapter 4) the percent by which common ion reduced dissolution rate was greater for the lased material, in addition to the lased samples having a two times lower initial dissolution rate to begin with.

**Molar solubility calculations in the presence of fluoride**

As mentioned in Chapter 4, a more meaningful comparison than solubility products is the comparison of molar solubilities in various media. Solubility products indicate whether something will occur; molar solubility is a measure of expected rate. Solubility calculations that include the effects of salivary fluoride by assuming that the ion activity product in the presence of fluoride corresponds to fluorapatite stoichiometry are shown in Table 6.2. Those calculations demonstrate the expected efficacy of laser irradiation in protecting the teeth against *in vivo* acid challenges.

For purposes of these calculations, resting saliva was assumed to contain 10 mM acetate buffer at pH 6.6, with ionic strength equal to 0.15M. An acid challenge was assumed to be equivalent to introducing additional acetic acid into this baseline resting saliva. A variety of other assumptions have been used in model calculations, and similar conclusions to those summarized below have resulted in each case.

First, as concluded from Table 4.4, the reduction of the dissolution force controlling ion activity product from $10^{-120}$ to $10^{-130}$ causes the threshold pH at which dissolution can occur to be lowered from 5.49 to 4.76; the quantity of acetic acid needed to reach the threshold pH is increased 5-fold, from 1.68 mM to 7.88 mM; and the enamel solubility at pH 4.5 is decreased 5-fold. Second, by comparing Table 6.2 with 4.4, in the presence of only 0.01ppm of fluoride, the expected effect of lasing is even more dramatic: the threshold
Table 6.2 Calculated solubility of unlased (pK\textsubscript{FAP}=120) and lased (pK\textsubscript{FAP}=130) HAP in synthetic saliva with low fluoride (0.01ppm)

<table>
<thead>
<tr>
<th>Dissolution medium (0.01ppm F)</th>
<th>Solubility</th>
<th>Quantity of HAc to reach K\textsubscript{FAP}</th>
<th>pH at which the K\textsubscript{FAP} is reached</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>HAP</td>
<td>5.30 x 10^{-7}</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lased HAP</td>
<td>2.02 x 10^{-7}</td>
<td></td>
</tr>
<tr>
<td>Synthetic saliva(^1) (pH=6.6)</td>
<td>HAP</td>
<td>-8.63 x 10^{-5}</td>
<td>4.58 x 10^{-3}</td>
</tr>
<tr>
<td></td>
<td>Lased HAP</td>
<td>-1.30 x 10^{-4}</td>
<td>2.97 x 10^{-2}</td>
</tr>
<tr>
<td>Synthetic saliva (pH=5.5)</td>
<td>HAP</td>
<td>-5.72 x 10^{-5}</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lased HAP</td>
<td>-1.23 x 10^{-4}</td>
<td></td>
</tr>
<tr>
<td>Synthetic saliva (pH=5.0)</td>
<td>HAP</td>
<td>1.89 x 10^{-6}</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lased HAP</td>
<td>-1.04 x 10^{-4}</td>
<td></td>
</tr>
<tr>
<td>Synthetic saliva (pH=4.5)</td>
<td>HAP</td>
<td>1.42 x 10^{-4}</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lased HAP</td>
<td>-5.76 x 10^{-5}</td>
<td></td>
</tr>
<tr>
<td>Synthetic saliva (pH=4.0)</td>
<td>HAP</td>
<td>4.37 x 10^{-4}</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lased HAP</td>
<td>4.67 x 10^{-5}</td>
<td></td>
</tr>
</tbody>
</table>

1. Synthetic saliva, 0.01ppm F, 1.5 mM Ca, 10 mM PO4, 10 mM acetate, ionic strength adjusted to 0.15 by NaCl, pH adjusted to 6.6 by adding HAc.
pH is reduced from 5.01 to 4.18; the quantity of acid needed to reach the threshold pH is increased over 6 times from 4.58 mM to 29.7 mM and the lased material does not dissolve at all at pH 4.5. At pH 4.0, the solubility for the lased material would be a factor of 10 lower than unlased in the presence of 0.01 ppm fluoride.

These calculations show that very modest amounts of fluoride can protect lased enamel from even a vigorous acid challenge. In fact no dissolution whatsoever is expected at pH 4.5 from enamel crystallites that have been annealed and behave as though their solubility product were $10^{-130}$, so long as there is as little as 0.01M fluoride present.

**Discussion**

Restraint should be exercised in the quantitative interpretation of the data reported here. Each of these density profiles represents the cumulative effect of a 24 hr exposure of enamel to a given acid challenge. There is no information on the time course of the mineral loss in any of these experiments. For example, a reduction of 50% in the amount of mineral lost in 24 hr could represent any scenario from a 50% reduction in rate maintained throughout the duration of the experiment to a 100% reduction for 12 hr followed by 12 hr of loss at the same rate as the control. The truth is likely somewhere in between these extremes. A 50% reduction in cumulative mineral loss while more reduction in IDR ascribed to laser treatment is likely the result of a much greater reduction in rate that persists until the more acid resistant enamel is dissolved, after which dissolution might occur at a rate similar to that of untreated enamel. Such a time course of mineral loss might occur whether the laser effect was that of a complete transformation of the mineral through only the first few microns of enamel or that of a partial transformation of the mineral through a much broader region of the enamel.

The effects of a chemical agent applied as a pretreatment might be expected to have a time course similar to that suggested above for the effect of laser treatment. Effectiveness would decrease and eventually vanish as the agent is leached from the enamel. When an
agent is continuously present as in the experiments done here, the situation is quite different. In this case there is no reason to expect that the effectiveness should decrease with time. In fact, the effectiveness might actually increase with time as the agent diffuses deeper into the enamel.

As these comments suggest, there is very little actually known and much to be learned about time course of lesion development and hence the mechanism for its inhibition under the conditions studied here. Nonetheless, it is clear that there is increased protection from acid challenge with increased laser irradiation (within the limited conditions studied here) and that the combination of laser pretreatment and each of the chemical agents studied is much more effective than either laser or chemical agent alone.

There are two major differences between the results reported here and those that might be clinically attainable. The first concerns the levels of laser irradiation used in these studies. The 65J/cm$^2$ level used in most of the experiments has been shown to not result in detectable histological damage to teeth and would likely be clinically acceptable. At 130J/cm$^2$ the situation is completely different, there is visible damage to the outer enamel in the form of a brown discoloration. Thus the results presented here must be regarded only as indicative of what may be feasible. Further exploration of laser irradiation conditions will be required to find conditions in which this same effectiveness is achieved with a lower laser energy.

The second difference between these studies and clinical situations is in the severity of the conditions used here. Although the pH encountered during an in vivo acid challenge may drop to 4.5 or even 4.0, there will normally be calcium and phosphate concentrations of several millimoles per liter in the saliva and plaque fluid to which tooth surfaces are exposed. The conditions used here were chosen for experimental convenience and to demonstrate that the combination of laser and chemical agents could be effective in improving the resistance of enamel to acid challenge. It should be noted, however, that
these conditions not only overestimate the damage that might result from an acid challenge, they also underestimate the effectiveness of the laser treatment in combatting this challenge.

**Conclusion**

The CO2 laser effect can be enhanced by chemical inhibitors, similar to the effect seen with high temperature treatment of synthetic hydroxyapatite. We can conclude the following:

1. The dissolution inhibition effect of CO2 laser can be enhanced by those chemical inhibitors (EHDP, DAC, and fluoride).

2. After laser treatment at higher energies (130 J/cm²) the effects of DAC and/or EHDP are even more dramatic. No dissolution is observed via quantitative microradiography even after 24 hr of exposure to a sink buffer solution. Such a solution removes about 150 microns of enamel from unlased control samples in the absence of these agents. The effects of laser irradiation and EHDP/DAC are synergistic in this case.

3. The initial dissolution rate reduction in the presence of fluoride is consistent with the two site model for HAP - the laser irradiation anneals away the more labile site #1 crystal and dissolution is controlled by the less soluble site #2 crystal.
CHAPTER 7

INFRARED PYROMETER MEASUREMENT OF THE SURFACE TEMPERATURE DURING LASER IRRADIATION

Introduction

As described previously, the purpose of this thesis is to investigate the use of laser irradiation as a method for the prevention of dental caries. The mechanism for this effect is assumed to result from the thermal effects of laser irradiation (Chapter 4). A technique for solving the appropriate heat conduction model has already been developed (Chapter 5). This model allows the temperature profiles in the tooth to be calculated as a function of time and position. However, it is necessary to measure the surface temperature during irradiation and subsequent cooling to confirm the accuracy of these model calculations. The measurement of surface temperature by an infrared thermometer has previously been reported by others (Launay et al., 1987; Nelson et al., 1987). Infrared pyrometer temperature measurements will enable us to test the thermal hypothesis for the effect of laser irradiation.

The objectives of this chapter are as follows:

1. Determine the surface temperature change of synthetic apatite (≈2% CAP) and enamel slabs when exposed to CO₂ laser irradiation.

2. Quantify the heat loss from the surface by use of appropriate heat transfer coefficients.

3. Compare the experimentally measured surface temperatures with calculated temperatures.
Materials and methods

Laser treatment of human enamel, C-HAP

The human enamel block and C-HAP pellets were positioned in a platinum crucible in a covered porcelain container as shown in Figure 4.1. The enamel and apatite pellet surfaces were irradiated by a continuous wave CO₂ laser (LaserSonics) with the power set at 50W and 25W for total periods of 1 to 8 sec from a distance of 37 cm. The laser beam width was 14 mm in diameter, which is slightly greater than the diameter of the samples used for subsequent dissolution tests. Another set of experiments was done by continuously heating the sample in air until a steady state temperature was reached. A schematic diagram of the experimental setup is provided in Figure 7.1.

Surface temperature measurement during laser irradiation

During the irradiation process, surface temperature measurements were collected at 1 sec intervals with a Modline Plus Series 7000 Infrared Pyrometer (Ircon, Niles, IL). This is a non-contact temperature measuring system. It measures temperature indirectly by measuring infrared energy radiated by the sample since the intensity of this radiation varies with the temperature of the object. The Series 7000 Infrared Pyrometer consists of a sensor and an indicator/processor linked by a signal cable as shown in Figure 7.2. The indicator/processor processes the incoming signals and provides a temperature display directly with a response time of 0.01 sec.

Theoretical surface temperature by model calculations

The surface temperature in the enamel slab and synthetic materials as a function of time can be described by the following partial differential equation, where the first term on the right hand side accounts for heat diffusion in the slab (pellet) and the second term accounts for heat production via absorption of laser radiation.

\[
\frac{\partial T}{\partial t} = \frac{\partial}{\partial x} \left( \alpha(T) \frac{\partial T}{\partial x} \right) + \beta(T) M(\lambda, T) Q \exp \left( -M(\lambda) x \right)
\]
Figure 7.1 Schematic representation of the apparatus for measuring steady state surface temperature during laser irradiation of CAP pellets
Figure 7.2 Basic pyrometer setup for surface temperature measurement.
This equation along with appropriate boundary conditions has been solved by the method of Laplace transforms as discussed in Chapter 5. All quantities except the heat transfer coefficients are well established (Brown et al., 1970). In order to facilitate the calculation, the semi-infinite solid approximation was applied here (Carslaw et al., 1959). All the calculations were done by neglecting the heat loss from the surface \( h_1 = h_2 = 0 \). Actually there are radiative and convective heat losses from the exposed surfaces. We therefore expect the measured temperature to be lower than the calculated temperature. Also neglected here are the cooling effect due to blood flow and heating effect due to metabolism (Boehm et al., 1975), but those effects are extremely small in magnitude compared to laser heating.

In order to quantify the heat loss from the surface, the equilibrium temperature profile is very important since it relates directly to the heat transfer coefficient. The equilibrium temperature profile resulting from continuous heating can be described by the following steady-state ordinary differential equation

\[
0 = \frac{\partial}{\partial x} \left( \alpha(T) \frac{\partial T}{\partial x} \right) + \beta(T) M(\lambda, T) Q \exp \left( - M(\lambda) x \right)
\]

subject to the same boundary conditions as shown in Chapter 4

\[
\text{at } x = 0 \quad \frac{\partial T}{\partial x} = \frac{h_1}{k} T
\]

\[
\text{at } x = L \quad \frac{\partial T}{\partial x} = -\frac{h_2}{k} T
\]

This kind of second order ordinary differential equation can be solved by the method of "particular integrals" (Jenson et al., 1977). The particular integral for this equation can be
obtained by the method of "undetermined coefficients" (Jenson et al., 1977) and the resulting complete solution is

\[ T = - \frac{\beta M(\lambda) Q_0}{\alpha M(\lambda)^2} \left[ \exp \left( - M(\lambda) x \right) + (\text{const} \, 1) x + (\text{const} \, 2) \right] \]

Substitution of the above expression into the two boundary conditions gives two linear equations with two unknowns (i.e. "const1" and "const2"). Simple algebra was applied here solving for these two constants and the solutions can be expressed as follows:

\[
\text{const 1} = \frac{\left[ \frac{\beta Q_0}{\alpha M^2} \left( \frac{h}{k} - M \right) \right] \left( \frac{h}{k} \right) - 1 + \exp \left( - M L \right)}{\frac{h^2}{k^2 L}}
\]

\[
\text{const 2} = \frac{\left[ \frac{\beta Q_0}{\alpha M^2} \left( \frac{h}{k} - M \right) \right] \left( - 1 + \exp \left( - M L \right) + \frac{h}{k} L \right)}{\frac{h^2}{k^2 L}}
\]

The heat transfer coefficients for both sides of the pellet were assumed to be the same since the major mechanism for heat loss from the surface is governed by convection.

Results and discussion
Surface temperature profiles by pyrometer measurement and model calculation

The computer simulated time-dependent surface temperature for each laser case (assuming no heat loss from the surface) is shown by solid lines in Figures 7.3-7.8. Experimentally measured surface temperatures are shown by closed circles overlapped on
Figure 7.3 Surface temperature profile for lased CAP pellet (25W, 4sec=65J/cm²)

(----) theoretical

(●) experimental
Figure 7.4 Surface temperature profile for lased CAP pellet (50W, 2 sec=65J/cm²)

(——) theoretical  (●) experimental
Figure 7.5 Surface temperature profile for lased CAP pellet (25W, 8sec=130J/cm²)

(---> theoretical  (●) experimental
Figure 7.6 Surface temperature profile for lased CAP pellet (50W, 4sec=130J/cm²)
(---) theoretical  (●) experimental
Figure 7.7 Surface temperature profile for lased human enamel (25W, 8sec=130J/cm²)

(----) theoretical  (●) experimental
Surface Temp (50W4S)

Human enamel

Figure 7.8 Surface temperature profile for lased human enamel (50W, 4sec=130J/cm²)

(----) theoretical (●) experimental
the same figure. Each temperature point is the average for three runs; the standard error for each set of runs was approximately ±40°C.

Since the minimum detectable temperature for the infrared pyrometer is 235°C, only limited experimental data can be collected while the temperature is rising. No experimental data can be collected after peak temperature because the temperature decays to a value lower than the minimum detectable temperature (235°C) within a very short period of time.

In general the computer results are higher than the experimental results (Figures 7.3-7.8). Part of the discrepancy is due to neglecting heat losses from the surface, but this can be corrected later by accounting for these losses. Another possible error is caused by taking the absorptivity of the sample as unity with respect to CO₂ laser wavelength (10.6 micron). No measurements have been made for absorptivity in the infrared range (Boehm and Gregory, 1975), but it is widely accepted that the absorptivity should be greater than 0.80 (Boehm et al., 1975). Calculations show that a difference of less than 10°C would result from using 0.80 as absorptivity instead of 1.0.

Determination of heat transfer coefficient

Heat loss from the surface occurs through convection and also by radiation. It is expected that heat loss from radiation will dominate at sufficiently high temperature because of its T⁴ dependence. The sample in the air was brought to a steady state temperature with the heating laser as shown in Table 7.1. At this point, the rate of energy absorption will be equal to the energy loss from both sides via convection and radiation. Thus the total heat transfer coefficient (h₁₀₅) is directly calculable at this temperature by fitting the equilibrium temperature to the steady state solution of the heat diffusion equation as shown in Table 7.1 and Figures 7.9-7.12. Using MINSQ software on an IBM computer, the best fitted total heat transfer coefficient (h₁₀₅) can be obtained. Furthermore, the total heat transfer coefficient can be decomposed to two parts as follows:
Table 7.1  Equilibrium front surface temperature reached and fitted total heat transfer coefficient under different continuous laser irradiation

<table>
<thead>
<tr>
<th>Power</th>
<th>Equilibrium Temperature (°C)</th>
<th>( h_{\text{tot}} ) (cal/sec/cm²/°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20W</td>
<td>324°C</td>
<td>0.005649</td>
</tr>
<tr>
<td>30W</td>
<td>485°C</td>
<td>0.005659</td>
</tr>
<tr>
<td>40W</td>
<td>535°C</td>
<td>0.006989</td>
</tr>
<tr>
<td>50W</td>
<td>585°C</td>
<td>0.008127</td>
</tr>
</tbody>
</table>
Figure 7.9 Equilibrium front surface temperature for lased CAP pellet (20W)

(- - -) theoretical  (●) experimental
Equilibrium Temp (30W, Front)

Figure 7.10 Equilibrium front surface temperature for lased CAP pellet (30W)

(- - - ) theoretical  (● ) experimental
Figure 7.11  Equilibrium front surface temperature for lased CAP pellet (40W)
(----) theoretical  (○) experimental
Figure 7.12  Equilibrium front surface temperature for lased CAP pellet (50W)

(----) theoretical  (●) experimental
\[ h_{\text{tot}} = h_c + \sigma \frac{(T + 273)^4}{T} \]

where

- \( h_c \) = convection heat transfer coefficient (cal/sec/cm\(^2\)/°C)
- \( \sigma \) = Stefan-Boltzmann constant (1.355x10\(^{-12}\) cal/sec/cm\(^2\)/°K\(^4\))
- \( T \) = temperature (°C)

The best fit value of \( h_c \) is -0.005. The significance of this value can be realized by comparing the calculated temperature profile for both cases (\( h_{\text{tot}} = 0 \) and \( h_{\text{tot}} = 0.005 \)). One typical comparison can be made from Figure 7.3 (\( h_{\text{tot}} = 0 \)) and Figure 7.13 (\( h_{\text{tot}} = 0.005 \)), where more than 50°C difference in peak temperature calculation was observed. This indicated that the insulated boundary assumption is not a good approximation. Heat loss by radiation is expected to play a role at high temperature which is consistent with the increased total heat transfer coefficient at high temperature as shown in Table 7.1. A final point to be mentioned here is that the heat transfer coefficient obtained by heating of C-HAP pellet can also be applied to human enamel, since the heat transfer coefficient is an environmental parameter and should be material independent.

**Surface temperature profiles by pyrometer measurement and model calculations corrected for the heat transfer coefficient**

The computer simulated time dependent surface temperature for each laser case corrected for heat transfer coefficients are shown by solid lines in Figures 7.13-7.18. Experimentally measured surface temperature were again shown by closed circles overlapped on the same figure. The difference between experimental and theoretical temperatures was greatly reduced by inclusion of heat transfer coefficients in the calculation.
Figure 7.13  Surface temperature profile for lased CAP pellet (25W, 4sec=65J/cm²)
(----) theoretical  (●) experimental
Figure 7.14 Surface temperature profile for lased CAP pellet (50W, 2sec=65J/cm²)

(---) theoretical  (●) experimental
Figure 7.15  Surface temperature profile for lased CAP pellet (25W, 8sec=130J/cm²)
(-----) theoretical  (●) experimental
Figure 7.16 Surface temperature profile for lased CAP pellet (50W, 4sec=130J/cm²)
(---- ) theoretical
(● ) experimental
Figure 7.17  Surface temperature profile for lased human enamel (25W, 8sec=130J/cm²)
(----) theoretical  (●) experimental
Figure 7.18  Surface temperature profile for lased human enamel (50W, 4sec=130J/cm²)
(----) theoretical  (●) experimental
Conclusions

After computer simulated surface temperature profiles were compared with experimentally measured surface temperatures, we reached the following conclusions:

1. The thermal hypothesis for the effect of laser irradiation proposed in Chapter 4 and 5 was supported by the temperature data measured in this chapter. The insulated boundary assumption applied in Chapter 5 is not a good assumption, and the heat transfer coefficient should be considered for each calculation in order to get more accurately calculated temperatures. However, the purpose of all the calculations in Chapter 5 is for qualitatively comparing temperature profiles and acid resistance. All the conclusions in Chapter 5 were derived from relative comparisons and not absolute temperatures. The insulated boundary assumption has the advantage of providing a upper limit for the real temperature and provides more conservative estimate of clinical safety.

2. The heat loss from the surface during laser irradiation was quantified by heat transfer coefficients calculated from steady state surface temperatures. Both convection and radiation processes contribute to the heat loss mechanism. Radiation heat loss plays an important role at the higher temperatures encountered here.

3. Discrepancies between theoretical and experimental temperature profiles can be reduced by accounting for heat loss from the surface.
CHAPTER 8

CONCLUSIONS

The ultimate goal in the laser dental research area at the University of Utah is to develop an effective and safe method of laser treatment to prevent dental decay. This thesis is a first step in proceeding towards this goal, and it differs from previous investigations in several ways. The empirical treatment of teeth with laser irradiation had previously been shown to be promising as a potential anticaries therapy by many other investigators. However, the performance of the laser can not be optimized until the system is well understood. This thesis has demonstrated the feasibility of laser treatment in reducing caries susceptibility of tooth enamel, and has also established laser operating parameters that optimize the effectiveness of such treatments with the continuous wave CO₂ laser. The following were accomplished:

1. We proposed that the reason for the caries inhibition effect of CO₂ laser irradiation is that the apparent solubility of enamel is reduced. The dissolution rate driving force for human enamel was changed from an apparent solubility product of about 10⁻¹²⁰ to an ion activity product of about 10⁻¹³⁰. This is similar to the effect seen with high temperature treatment of synthetic hydroxyapatite samples and is consistent with the annealing away of the more labile dissolution site #1, resulting in dissolution controlled by the less labile site #2. It is further suggested that the effectiveness of laser irradiation can be explained solely on the basis of thermal effects. It was also suggested that temperatures of 400-500°C are sufficient to anneal enamel crystallites so that their apparent solubility is decreased.
2. The temperature profile developed in the enamel can be related to the acid resistance of that enamel. The "effectiveness" of laser treatment can be improved by varying the total energy density and the power delivery pattern based on the thermal hypothesis proposed in Chapter 4. This thermal hypothesis was confirmed by experimental surface temperature measurement.

3. There are synergisms between laser treatment (130J/cm²) and chemical inhibitors. The "effectiveness" of laser treatment can be enhanced with these chemical inhibitors. Complete (100%) inhibition of enamel dissolution can be achieved by this combined treatment.

These conclusions indicate that we are not far away from real clinical application of laser treatment to prevent dental caries. Several pathways for future research are as follows:

1. The irradiation conditions investigated in this study are very unlikely to be optimal. The 130 J/cm² laser irradiation, although very effective, leads to browning of teeth and would not be clinically acceptable. Further studies with other wavelengths and irradiation conditions could very well lead to more effective and clinically acceptable laser treatment regimens. The 10.6 micron wavelength was chosen solely because of its availability. Several wavelengths which would be of interest are listed in Table 8.1. Those wavelength were chosen to test out two questions: 1) whether there is a specific wavelength at which the annealing away of more labile dissolution sites can occur more easily; that is, is it better to selectively stimulate the phosphate group, or the carbonate group, or both? and 2) Is it better to pick a less efficiently absorbed wavelength to broaden the zone of annealing, especially for high power short pulses? The recent development of the free electron laser offers the possibility of choosing from a continuously variable range of wavelengths and a variety of pulse patterns and thereby greatly increases the opportunity for finding truly optimal conditions for laser treatment.
Table 8.1 Laser irradiation wavelengths selected for future study

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>Characteristic</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.9 μm</td>
<td>carbonate absorption maximum</td>
</tr>
<tr>
<td>7.7 μm</td>
<td>absorption minimum</td>
</tr>
<tr>
<td>9.7 μm</td>
<td>absorption maximum due to phosphate and carbonate</td>
</tr>
<tr>
<td>10.6 μm</td>
<td>CO₂ laser wavelength for comparison with existing data</td>
</tr>
<tr>
<td>13.0 μm</td>
<td>absorption minimum</td>
</tr>
<tr>
<td>17.0 μm</td>
<td>phosphate absorption maximum</td>
</tr>
</tbody>
</table>
2. Although the one-dimensional heat conduction model has been useful in establishing the approximate experimental temperatures, more accurate simulation results could be obtained by considering the two-dimensional heat transfer equation as follows:

$$\frac{\partial T}{\partial t} = \frac{\partial}{\partial x} \left( \alpha(T) \frac{\partial T}{\partial x} \right) + \frac{\partial}{\partial y} \left( \alpha(T) \frac{\partial T}{\partial y} \right) + \beta(T) M(\lambda, T) Q \exp \left( - M(\lambda) x \right)$$

The above two-dimension partial differential equation can not be solved by Laplace transform and other numerical techniques must be employed. Different physical properties (heat capacity, thermal conductivity, and thermal diffusivity) of enamel and dentin could also be taken into consideration. The possible temperature dependence of each physical properties should also be considered.

3. Even greater effectiveness might be achieved by combinations of the chemical agents considered in this study. We might imagine that various combinations of these agents (EHDP, DAC, and fluoride) working together by their separate mechanisms would be even more effective.
APPENDIX

THEORETICAL EQUILIBRIUM CALCULATION

All the calculations were carried out by a commercially available computer program, EQUIL (Micromath Scientific Software, Salt Lake City, UT 84121). The following calculation is presented as an example that could explain all the theoretical calculations in Table 4.4 and Table 6.2:

Example calculation: Table 4.4 - Synthetic Saliva (pH=6.6)

1. Construct dissolution medium:

   Resting saliva were assumed to contain 1.5 mM Ca, 1.0 mM PO4, and 10 mM acetate buffer. The ionic strength was adjusted to 0.15 by adding NaCl. The pH was adjusted to 6.6 by adding HAc. The equilibria of all the species in the system must be considered as shown in Table A.1 and Table A.2. The whole equilibrium system was transferred to a set of nonlinear algebraic equations and than solved by an iterative computer algorithm (Fox, 1977).

2) Calculate molar solubility:

   The molar solubilities were calculated by ionic activity product

   \[ K_{\text{HAP}} = a_{\text{Ca}}^{10} a_{\text{PO4}}^{6} a_{\text{OH}}^{2}, \]

   where a's are ionic activities. The \( K_{\text{HAP}} \) values were assumed to be \( 10^{-120} \) for HAP and \( 10^{-130} \) for lased HAP. These two values were used as constraints for the previously constructed dissolution medium. One of these constraints was added to
Table A.1 Chemical equilibria and equilibrium constants used in the equilibrium calculation

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Equilibrium Constant (pKa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{H}_3\text{P}O_4$ $\leftrightarrow$ $\text{H}_2\text{P}O_4^-$ $+$ $\text{H}^+$</td>
<td>2.15</td>
</tr>
<tr>
<td>$\text{H}_2\text{P}O_4^-$ $\leftrightarrow$ $\text{HPO}_4^{-2}$ $+$ $\text{H}^+$</td>
<td>7.20</td>
</tr>
<tr>
<td>$\text{HPO}_4^{-2}$ $\leftrightarrow$ $\text{PO}_4^{-3}$ $+$ $\text{H}^+$</td>
<td>12.35</td>
</tr>
<tr>
<td>$\text{HAc}$ $\leftrightarrow$ $\text{Ac}^-$ $+$ $\text{H}^+$</td>
<td>4.76</td>
</tr>
<tr>
<td>$\text{CaAc}^+$ $\leftrightarrow$ $\text{Ca}^{2+}$ $+$ $\text{Ac}^-$</td>
<td>1.26</td>
</tr>
<tr>
<td>$\text{CaPO}_4^-$ $\leftrightarrow$ $\text{Ca}^{2+}$ $+$ $\text{PO}_4^{-3}$</td>
<td>6.46</td>
</tr>
<tr>
<td>$\text{CaHPO}_4^-$ $\leftrightarrow$ $\text{Ca}^{2+}$ $+$ $\text{HPO}_4^{-2}$</td>
<td>2.74</td>
</tr>
<tr>
<td>$\text{CaH}_2\text{PO}_4^+$ $\leftrightarrow$ $\text{Ca}^{2+}$ $+$ $\text{H}_2\text{P}O_4^-$</td>
<td>0.70</td>
</tr>
<tr>
<td>$\text{HF}$ $\leftrightarrow$ $\text{F}^-$ $+$ $\text{H}^+$</td>
<td>3.17</td>
</tr>
<tr>
<td>$\text{H}_2\text{O}$ $\leftrightarrow$ $\text{OH}^-$ $+$ $\text{H}^+$</td>
<td>13.83</td>
</tr>
</tbody>
</table>
### Table A.2 Ionic activity coefficient for each species used in the calculations

<table>
<thead>
<tr>
<th>Species</th>
<th>Ionic activity coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>$H_3PO_4$</td>
<td>1.00</td>
</tr>
<tr>
<td>$H_2PO_4^-$</td>
<td>0.69</td>
</tr>
<tr>
<td>$HPO_4^{2-}$</td>
<td>0.19</td>
</tr>
<tr>
<td>$PO_4^{3-}$</td>
<td>0.02</td>
</tr>
<tr>
<td>$Cl^-$</td>
<td>0.64</td>
</tr>
<tr>
<td>$HAc$</td>
<td>1.00</td>
</tr>
<tr>
<td>$Ac^-$</td>
<td>0.69</td>
</tr>
<tr>
<td>$HF$</td>
<td>1.00</td>
</tr>
<tr>
<td>$Ca^{2+}$</td>
<td>0.28</td>
</tr>
<tr>
<td>$CaAc^+$</td>
<td>0.68</td>
</tr>
<tr>
<td>$CaPO_4^-$</td>
<td>0.69</td>
</tr>
<tr>
<td>$CaHPO_4^-$</td>
<td>1.00</td>
</tr>
<tr>
<td>$CaH_2PO_4^+$</td>
<td>0.69</td>
</tr>
<tr>
<td>$F^-$</td>
<td>0.69</td>
</tr>
<tr>
<td>$H^+$</td>
<td>0.88</td>
</tr>
<tr>
<td>$OH^-$</td>
<td>0.79</td>
</tr>
</tbody>
</table>
the original nonlinear equation before solving. The whole equation system was solved by the same computer algorithm as previously described (Fox, 1977).

3) *Calculate threshold pH*:

The molar solubilities calculated were negative under the solution condition (synthetic saliva, pH=6.6). Negative solubility is indicative of a supersaturated solution which means HAP does not dissolve at the above dissolution medium. It is interesting to calculate the necessary pH value (threshold pH) for HAP to dissolve. The $K_{HAP}$ values were again used as constraints in solving for the unknown pH.

The following calculation is presented as an example of the theoretical calculations in Table 4.1:

Example calculation: Table 4.1 - 3 mM Ca, $pK_{HAP}=120$

1. **Construct dissolution medium:**
   Same as above (for both sink and 3 mM Ca common ion conditions).

2. **Calculate amount dissolved:**
   Same as molar solubility calculation (for both sink and 3 mM Ca common ion conditions)

3. **Calculate % suppression:**
   The % reduction can be obtained by the ratio of two molar solubilities (the solubility under sink condition was assumed to be 100%).
REFERENCES


