University of Alberta

Argon Laser Induced Changes to the Carbonate Content of Enamel

by

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Abstract

The argon laser (AL) can be used to cure orthodontic brackets onto teeth in significantly less time than conventional curing lights (CCL). As well, it has been shown that the argon laser seems to impart a demineralization resistance to the enamel. The purpose of this study was to use modern surface science techniques to ascertain if this demineralization resistance is possibly a result of a decrease in the carbonate content of enamel.

Eleven mandibular third molars previously scheduled for extraction were collected. The teeth were sectioned in two and randomly assigned to either the argon laser (250 mW/cm^2, 10 s) or the control (no treatment) group. The sections were then analyzed using X-Ray photoelectron spectroscopy (XPS) and time of flight secondary ion mass spectrometry (ToF-SIMS). These surface science techniques can be used to detect changes in the carbonate content of the enamel. The results showed no statistically significant change in the carbonate content of enamel after AL irradiation ($p>0.05$). Thus, it is suggested that any demineralization resistance imparted to the enamel surface by AL irradiation is not due to alterations in carbonate content.

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Table of Contents

List of Tables

List of Figures

Appendices

List of Nomenclature

Absorption: The incorporation of the laser energy by the intended target tissue. This effect is the usual desirable effect, and the amount of energy that is absorbed by the tissue depends on the tissue characteristics, such as pigmentation and water content, and on the laser wavelength and emission mode.

Auger effect: A sample bombarded by electrons or x-rays will eject core electrons from a level E_x in atoms in a region of the sample up to 1 μm deep. The core hole is then filled by an internal process in the atom whereby an electron from a level E_y falls into the core hole with the energy balance taken by a **third electron** from a level E_y'. This last electron, called an **Auger electron** after **Pierre Auger** is then ejected from the atom with an energy Ea, given approximately by the equation: $E_a = E_y' + E_y - E_x$.

Birefingence: The resolution or splitting of a light wave into two unequally reflected or transmitted waves by an optically anisotropic medium such as calcite or quartz, also referred to as double refraction.

Coherency: A property unique to lasers. The light waves produced by a laser are a specific form of electromagnetic energy. A laser produces light waves that are physically identical. They are all in phase with one another and have identical amplitude, that is, all the peaks and valleys are same size.

Collimation: Refers to the beam having specific spatial boundaries. These boundaries insure that there is a constant beam size and shape that is emitted from the laser cavity. An X-ray beam has the identical property.

Fluence: The energy incident on a surface expressed in Joules per centimeter squared (Joules/cm^2) = energy density. To calculate the energy density (J/cm^2), the output of the curing source (in mW/cm^2) and the duration of the exposure (in seconds) are used.

$$\textbf{Energy density} = \textbf{(Watts x seconds) / cm}^2 = \textbf{Joules / cm}^2 = \textbf{J / cm}^2$$

Monochromatic: In contrast to ordinary light produced by a table lamp, laser produce a monochromatic not a polychromatic light. It only has one specific wavelength and is very finely focused.

Chapter One: Introduction and Literature Review

1.1 Introduction

Orthodontics requires the bonding of brackets to the teeth to subsequently move the teeth with the assistance of orthodontic wires. However, there are negative aspects of this bonding to the tooth structure. One of the most frequently encountered negative aspects of bracket bonding is the appearance of localized decalcification around the bracket base, referred to as a "white spot" lesion.[1] These white spot lesions are due to demineralization of the enamel by organic acids produced by cariogenic bacteria,[2] and are primarily caused by inadequate oral hygiene and poor diet control. Despite the advances in orthodontic materials and techniques in recent years, the development of decalcifications around the brackets during orthodontic treatment continues to be a problem. Nearly 50% of orthodontic patients exhibit clinically visible white spot lesions during treatment lasting approximately 2 years.[3] Preventing these lesions during treatment is an important concern for the orthodontist because the lesions are unaesthetic, unhealthy, and potentially irreversible.[3]

The argon laser (AL) is used to cure the composite material bonding the orthodontic bracket to the enamel surface. Several studies have shown that the treatment of enamel with an AL can make it more resistant to caries. [4-8] However, the question remains as to why the enamel surface is more resistant to caries after treatment with the AL. As well, these studies did not show that this increased caries resistance was permanent. Due to the length of orthodontic treatment (2 years or more), any changes must be somewhat permanent to have clinical implications. If it could be shown that the resistance of the enamel surface was due to an elemental chemical change, this

would demonstrate that the surface alterations could be considered permanent.

Several investigators have speculated that the increased caries resistance was due to decreased solubility of the enamel resulting from an alteration in the composition of the mineral phase.[9-11] In particular, the consensus of many studies is that carbonate incorporation into enamel is the main cause for shifts in the solubility product of biological apatites, making them more soluble than pure hydroxyapatite.[12] This increase in solubility due to the inclusion of carbonate makes the enamel more susceptible to a carious attack.

In view of the profound effects of carbonate on both the physical structure and chemical stability of dental enamel, the present study was undertaken to determine the concentration of carbonate in enamel before and after exposure to AL irradiation. The aim of this study was to demonstrate how time of flight secondary ion mass spectrometry (ToF-SIMS) and X-ray photoelectron spectroscopy (XPS) could be used to analyze AL induced changes in the carbonate concentration of enamel.

Gaining more information on how and why AL irradiation changes the enamel surface and confers protection against demineralization is necessary for this technology to have a widespread acceptance into clinical practice.[1]

1.2 Literature Review

1.2.1 The Role of Carbonate in Enamel

The carbonate ion is a polyatomic anion with the empirical formula CO_3^{2-} and a molecular mass of 60.01 daltons; it consists of one central carbon atom surrounded by three identical oxygen atoms in a trigonal planar arrangement (Fig 1.1). Many researchers believe that there is a connection between a raised level of carbonate in teeth and their susceptibility to caries.[13] For this reason carbonate has been called the "Achilles heel" of enamel.[13] The acid lability of the carbonate ion together with its adverse effect upon the crystallization of hydroxyapatite warrants special attention, because of all the ions present in the enamel mineral, carbonate will offer the least resistance to an acid attack on exposed tooth surface.[14]

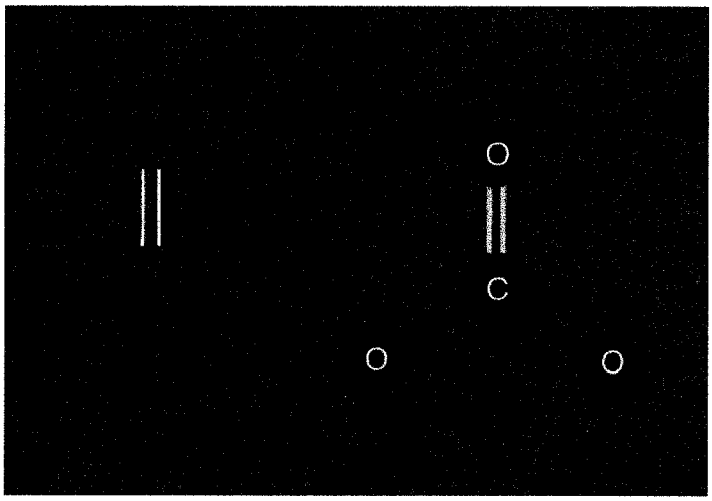

Fig. 1.1 Carbonate Ion [15]

The process of enamel matrix formation and calcification can be split into a number of phases; overall, the process is known as amelogenesis.[16] In the proliferative phase, preameloblast cells form. These differentiate (change their nature and function) in the presecretory phase to form ameloblast cells which secrete enamel matrix proteins (amelogenins and enamelins). The protein gel adjacent to the ameloblast is supersaturated in calcium and phosphate ions and carbonated hydroxyapatite is precipitated almost immediately. Once the ameloblasts stop secreting protein, there is a maturation phase during which the apatite crystals grow and the proteins dissolve or are resorbed. Ultimately, the ameloblasts withdraw, leaving apatite crystals stacked in rods (or prisms) with an enamelin-rich boundary layer between the rods. Once tooth formation is complete, the enamel forming cell is lost and no repair is possible.[17]

Enamel is the hardest biological substance in the human body and is a crystalline structured material consisting of both a mineral and an organic phase. The average size of the crystallites is about 30 nm thick by 60 nm wide and several microns long.[17] The mineral phase predominates (96 wt. %) and consists primarily of calcium hydroxyapatite ($Ca_5(PO_4)_3(OH))_2$. The remainder of the enamel is made up of 3% water and 1% organic matter including proteins and lipids. Pure hydroxyapatite (HA) (Fig. 1.2) does not occur on a macroscopic scale in biological systems. Enamel, dentine, cementum and bone are instead made up from a calcium-deficient and carbonate-containing apatite analogue.[18]

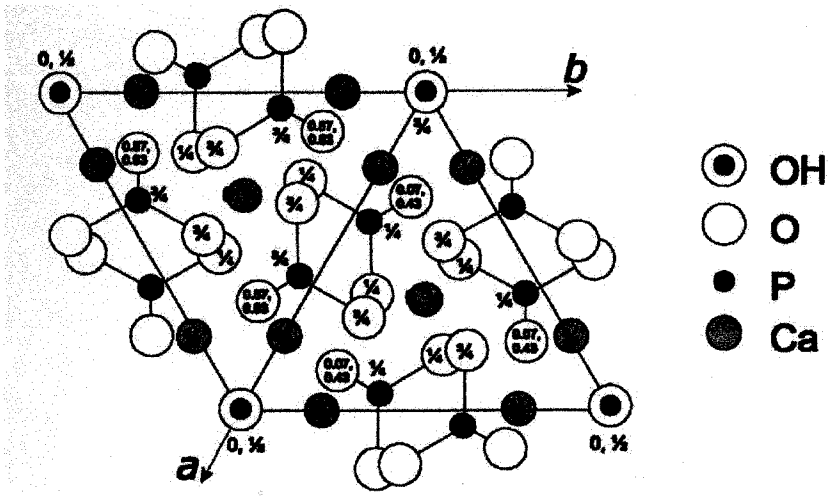

Figure 1.2 The idealized crystal structure of hydroxyapatite, viewed along the c-axis. Hydroxyapatite adopts the hexagonal structure with the OH groups ordered along the c-axis and Ca^{2+} occupying two different sites: positions on the corner of two 60° rotated triangles close in to the c-axis and positions at the corner of a hexagonal at a further distance from the c-axis. Pure Hydroxyapatite does not occur on a macroscopic scale in human enamel, instead made up from a Ca-deficient and carbonate containing apatite analogue. Carbonate can substitute for the OH groups, but this is only believed to occur on a minor scale. Instead, the planar CO_3^{2-} group mainly substitutes for the tetrahedral PO_4^{3-} (Jones FH. Surface Science Reports, 2001:86).

One of the more elusive problems in the chemistry of calcified tissues has been to explain satisfactorily the presence, chemical status and effects of carbonate. This ion is present in varying amounts in the surface of tooth enamel.[19] Carbonate is abundant in the body, and its presence in the mineral phase may depend on the metabolic activity of the cells near the forming hydroxyapatite crystallites.[20] When dental enamel is formed it is laid down by cells which are producing carbon dioxide. The higher the level of metabolism the more carbon dioxide will be present, and the greater will be the opportunity for the newly formed crystallites to acquire carbonate. The ameloblasts lay down enamel behind them, and as they reach what will be the surface of the enamel, they begin to slow their metabolism or die off. Thus, it would

be expected the percentage of carbonate in enamel should decrease as the surface is approached, this decrease has in fact been shown.[21]

In this way the carbonate, arising from the metabolizing cells, is incorporated in the forming enamel crystallites at the expense of phosphate groups (PO_4^{3-}), which can act as centres of secondary crystal growth. As a result of this substitution, the crystallites will lose phosphate groups and be less able to grow. The fact that the crystals are restricted in their accustomed pattern of growth could also help to explain the differences in morphology seen in carbonate apatites.[22] Increasing carbonate in apatites causes a reduction in crystallinity, reflecting both a reduction in crystallite size and increased crystal strain. The result will be enamel containing a greater proportion of weaker and smaller crystals which are more soluble. When carious or acid attack occurs these small crystals will be the first to dissolve and will carry with them their elevated level of carbonate. If this view is true, then one would expect to find smaller enamel crystals in subsurface enamel, where the carbonate content is higher than at the tooth surface. Such findings have been reported.[23]

The primary substitution in carbonated apatite vs. hydroxyapatite is the carbonate replacing the phosphate group, leading to a more acid soluble mineral. Planar CO_3^{2-} mainly substitutes for the tetrahedral PO_4^{3-} group (Fig. 1.3), which disrupts the crystal structure and weakens chemical bonds. Charge neutrality is thought to be maintained via calcium deficiency either by Ca^{+2} absences or Na^{+} substitution.[17] This further decreases crystal size, which increases the surface area for acid attack, and thus

increases solubility. Carbonate can also substitute for the OH^- groups, but this is only believed to occur on a minor scale. Many other elements can be included in the HA structure; metal cations can substitute for calcium, silicate can substitute for phosphate and halide ions can substitute for hydroxide. The effect of such substitutions can have a critical effect on bulk behaviour and/or surface chemistry, depending on whether substitution occurs throughout the apatite crystal or is limited to the surface region. However, of all the substitutions which can occur, the substitution of carbonate into the HA crystalline structure has the most impact on the caries resistance of enamel.[24]

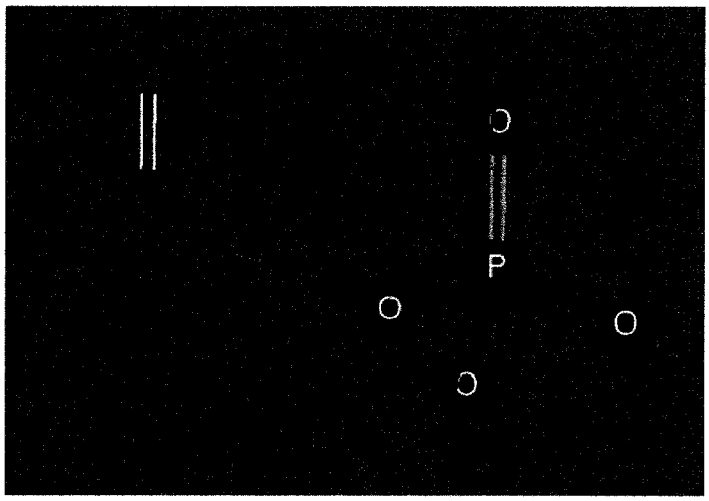

Fig. 1.3 Phosphate Ion [15]

It has been established that carbonate is lost faster than calcium and phosphate during enamel caries.[25] A general observation has been made that the presence of carbonate or bicarbonate in a calcifying medium inhibits the appearance of calcium phosphate as a solid phase and when calcification does occur the material is poorly crystalline. Apatites produced from bicarbonate containing media were found to be more soluble

at both slightly alkaline and acidic pH values.[26] These recent studies are in agreement with the previous work of *Hallsworth,*[24] who found that enamel rich in carbonate preferentially dissolves in the early carious lesion.

LeGeros and Tung[27] characterized synthetic apatites containing carbonate before and after two exposures to an acid buffer. This study demonstrated the following:

1) The extent of dissolution was directly proportional to the carbonate content of the apatite.
2) The extent of dissolution during the second exposure was much less than the first exposure.
3) Crystallinity and carbonate parameters differed before and after acid exposure. There was an increase in crystallinity (meaning an increase in the crystal size, which decreases the surface area exposed to acid) and lower carbonate concentration after the acid exposure.
4) There was also a decrease in the crystal strain.

These results suggest that the vulnerability of synthetic and biologic apatites to acid dissolution was largely due to their carbonate constituent, once it is reduced the apatite is more resistant to the next acid challenge.[28] The effect of the carbonate on the chemical instability of the carbonate containing apatites is a consequence of the effect of carbonate on the structural properties of the apatites, i.e. reduction in crystallite size, increase in crystal strain, and substitution of a weaker Ca-CO_3 bond for a stronger Ca-PO_4 bond. Thus, carbonate has profound effects on the chemical and physical stability of enamel in the oral environment. The carbonate substitutions

in the mineral crystal lattice disturb the structure and make if much more acid soluble than pure hydroxyapatite[29].

1.2.2 The Caries Process

Dental caries affects people universally. Since demineralization of the enamel is the precursor to dental caries, if you can reduce or prevent demineralization, you can reduce or prevent dental caries. Dental caries is the most common chronic disease in children and adolescents in the United States, as 85% of 17 year-old individuals have developed dental caries.[30]

Enamel decalcification or formation of "white spot" lesions during orthodontic treatment presents a significant problem in orthodontic patients (Fig. 1.4). Fixed orthodontic appliances complicate the removal of food debris that results in the accumulation of plaque. Several studies have found an increase of plaque around orthodontic appliances, and along with the plaque an increase in the number of *Streptococcus mutans* and *Lactobacillus* species.[31,32] Tooth demineralization and caries are caused by the acids (lactic, acetic, propionic, etc.) excreted by these bacteria as a product of their metabolism of carbohydrates from the food debris and plaque.[17]

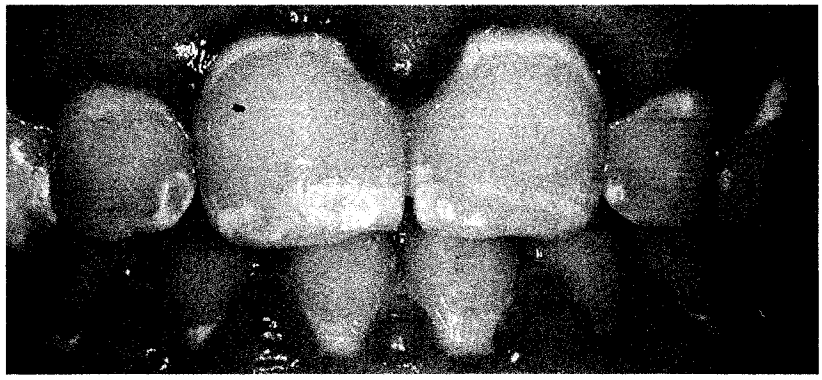

Fig. 1.4 "White spot lesions" from an orthodontic patient.
(Courtesy: University of Alberta; Graduate Orthodontics)

A tooth can be described as several distinct parts, as illustrated in Fig. 1.5. Teeth have to withstand a range of physical and chemical processes. These include compressive forces (up to ~700 N), abrasion and chemical attack due to acidic foods or products of bacterial metabolism. As protection, the dentinal body of the tooth is covered with an enamel layer. This can reach a thickness of over 2 mm at the cutting edges of the tooth.[17] Importantly, once enamel is damaged it has no ability to repair itself. Thus, a technique that could reduce chemical damage to the enamel surface would be invaluable.

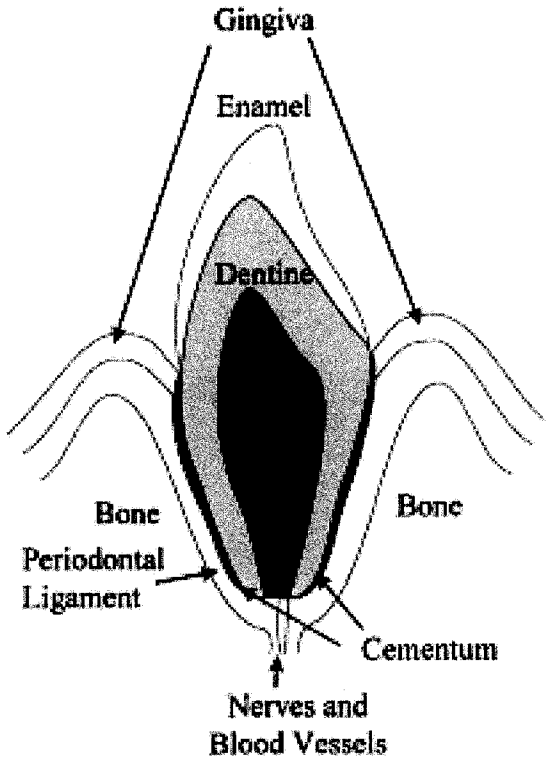

Fig. 1.5 Schematic representation of a tooth. The major features in the anatomy are displayed.[17]

The underlying physical process behind tooth decay or caries is essentially the dissolution of tooth mineral. Decay can develop from demineralized regions of enamel commonly called "white spot" lesions (Fig. 1.4). These lesions are caused by acids formed by bacterial fermentation of dietary sugars on the surface of the tooth. This leads to a fall in the plaque pH (more acidic), and dissolution of the mineral component of the tooth enamel. At pH<5.5, apatite can dissolve in the process known as demineralization.[33] Saliva can act as a natural buffer, serving to neutralize acid and restrict the dissolution process. Moreover, saliva is rich in calcium and phosphate ions. At pH > 5.5, apatite can be regained from salivary Ca^{+2} and PO_4^{3-}; this is the "remineralization" process.[33] The pH scale measures how acidic or basic a

substance is. The pH scale is logarithmic and as a result, each whole pH value below 7 is ten times more acidic than the next higher value.[15] Therefore, even a small shift in the acid resistance of enamel, could have a very large effect intraorally on the amount of acid needed to begin enamel dissolution.

In the caries process, enamel, the outermost layer of the tooth, is affected first. The carious lesions develop as a result of acids attacking the inorganic, apatite phase of the enamel. Dental enamel consists of mineral, protein, lipid and water. Even though the enamel is 96% by weight mineral, the water content of enamel is sufficient for diffusion of acids and other components into the tooth mineral. Subsequently, these acids can then dissolve portions of the mineral matrix of enamel, initiating the decay process.[29]

Under normal conditions, the demineralization process is balanced by remineralization due to diffusion of ions (Ca^{+2}, PO_4^{3-} and OH^-) from saliva, into enamel, when plaque pH returns to neutrality. However, if the demineralization process exceeds that of remineralization, an incipient carious lesion is formed. Both the macroscopic and microscopic changes in enamel associated with early carious lesions appear to be linked to specific chemical changes in the mineral, with little or no degradation of the organic matrix.[34] The process of demineralization can happen in as little as 4 weeks,[4] which gives the clinician little time to spot a potential problem before it is permanent.

Carbonate is the most abundant impurity in enamel and its role in dental caries has been vaguely implied.[27] As a result of this carbonate inclusion, the growth of the enamel apatite crystals is hindered to an extent dependent on the level of carbonate present, and the resultant enamel contains a proportion of small carbonate apatite crystals vulnerable to acid attack.[19] Carbonate substitution in the apatite structure results in a phase with a higher solubility product.[35] So at higher pH than 5.5, demineralization can occur more readily on a carbonated apatite, requiring less bacterial acid production to initiate the caries process. *Weatherell at al.*[20] found that the carbonated regions of apatite were indeed quicker to succumb to an acid attack. He found that in the initial region of carious attack, most of the enamel porosity is due to a relatively large ratio of the amount of carbonate lost, compared to the amount of lost mineral in general.

Additionally, studies have correlated the loss of carbonate from dental enamel with a reduction in acid dissolution.[36] Thus, at a given pH, the solubility of hydroxyapatite is approximately an order of magnitude below that of carbonated apatite,[37] making the pure hydroxyapatite more resistant to a bacterial acid attack.

Overall, the past literature indicates carbonate is an important element in enamel, and it has been implicated in the caries process in the following ways:

1) Carbonate is preferably lost in the early enamel caries process, suggesting the presence of carbonate rich apatitic phases.[38]

2) Carbonate in the enamel mineral was assumed to cause an increase in its solubility and therefore caries susceptibility.[26]
3) Carbonate and fluoride concentration in the enamel were assumed to be useful as a cariogenicity index, with high carbonate/low fluoride promoting caries.[27]
4) The observed preferential dissolution of the crystal core in enamel caries was speculated to be due to a carbonate rich mineral in the crystal core.[39]

If the argon laser (AL) could be used to bond orthodontic appliances onto teeth and also reduce the carbonate content of the enamel, it would prove to be extremely beneficial to clinicians and patients alike.

1.2.3 Use of the Argon Laser in Orthodontics

Composite resins are used extensively in orthodontics to bond the orthodontic brackets to the teeth. For many years, the standard process has been to use a conventional curing light (CCL), which hardens the composite resin and fixes the bracket to the tooth.

The word LASER is an acronym for Light Amplification by Stimulated Emission of Radiation. Dental lasers are named for the chemical elements, molecules or compounds that comprise the core, or active medium, that is stimulated.[1] In the case of the argon laser, the active medium is argon gas.

There are several advantages in using an argon laser for curing composite resins

versus the traditional curing light. The argon laser is monochromatic and emits light over a narrow band of wavelengths in the blue green spectrum (457.9-514.5 nm). This makes it ideally suited to polymerize composite resins, since the activator in composite resins, that initiates the polymerization process, responds to a wavelength of approximately 480nm. The difference between lasers is the wavelength of the emitted light, and this is dependent upon the laser medium, in this case argon gas.[40] Although conventional visible light curing units also emit energy centered around 480 nm, the energy is emitted over a much broader range, thus there is wasted energy. In addition, light from the argon laser is collimated, which results in more consistent power density over distance. In contrast, the power density of light reaching composite from a CCL decreases dramatically over distance, due to divergence of light from the source. Visible light curing units use bulbs, reflectors and filters which can degrade and decrease curing efficiency.[41] As well, the AL cures the composite resin in 10 seconds or less, versus 40 seconds for the CCL. This is a time savings of 30 seconds per tooth, which considering that there are often 28 teeth at a time bonded, can be a significant amount of time saved. Finally, it has been suggested that AL irradiated enamel is more resistant to demineralization. These are potential benefits of the AL curing of composite resins versus CCL, when used for orthodontic purposes.

Demineralization around orthodontic appliances is a problem. Suboptimal oral hygiene, long intervals between appointments and potentially poor patient cooperation with using fluoride dentifrices and mouth rinses calls for a compliance-free means of preventing tooth decay.[2] The AL is the only curing light which seems

to have the potential to reduce or even prevent demineralization. It would be of interest to know how the AL imparted this increased resistance, and if it is in fact due to a decrease in the amount of carbonate present in the enamel. Laser irradiation of teeth results in an interaction of light with the biological constituents of the dental hard substance. If it is absorbed by specific components of the dental enamel, the irradiated energy is converted directly into heat. This thermal effect is regarded as being the cause of microstructural and chemical changes in dental enamel following laser irradiation, and serves to explain the increase in acid resistance.[42] *Borggreven*[43] theorized that chemical not physical modifications were responsible for the increase in acid resistance.

Several theories have attempted to explain what chemical modifications reduced acid solubility of dental enamel after laser irradiation. In addition to reduced permeability of dental enamel following laser irradiation, the most widely accepted theory is based on the fact that bound carbonate is released when dental enamel is heated or irraditated.[44] A direct relationship has been demonstrated between carbonate loss in laser-irradiated enamel and reduced acid solubility.[45] This carbonate loss already begins following a temperature increase of 100 ^{o}C, increasing to the point of almost complete carbonate loss when the melting point is reached.[9,10,46] However, for orthodontic uses heat this high would not be practical, as it would damage the tooth, and surrounding tissues.

Many early studies that displayed chemical changes in the enamel used types of

lasers, or at power settings far higher than would be used in orthodontics. Several of these studies found a reduction in the carbonate content and increased demineralization resistance after exposure to a carbon dioxide laser.[9,47] However, the carbon dioxide laser operates on a different wavelength, 10.6μm irradiance as compared to 0.480μm irradiance (range of argon laser). It was found that lower energy densities were required for the higher wavelength (carbon dioxide) to achieve the equivalent demineralization and carbonate reduction.[47] Thus, the carbon dioxide laser is more readily absorbed by the enamel surface, however, this wavelength is of little use in curing orthodontic composites.

Other studies have used the AL to study changes in the enamel. *Ogaard*[48] found that argon laser irradiation is effective in reducing enamel decalcification during orthodontic treatment. However, in this study they used a power of 325 mW for 60 s with the argon laser. This power setting and curing time are more than are recommended for curing composites with an AL. The increased time defeats one of the main advantages of the AL.

In *Feuerstein et al.*[49] teeth which were clinically recognized as sound (not affected by caries, no developmental disturbances), were sliced longitudinally. Irradiated surface enamel regions (experimental) and adjacent sound surface enamel (control) were analyzed from the same tooth section. However, an ArF excimer laser was used in this case, which has a wavelength of 193 nm, and operates to remove enamel structure by a process of ablation. They found at fluences less than 450mJ/cm^2 some

changes were observed and this become more dramatic as the fluence increased. They speculated these changes may occur due to thermal processes. One of the main changes was a decrease in carbonate present in the apatite. They concluded that the ArF excimer laser can alter the chemical composition of enamel depending on the fluence used. The fluence (energy density) is calculated by using the following equation: Fluence (J/cm^2) = energy (W) x exposure time (seconds). With ablation a sufficient burst of energy must be transmitted to the enamel over a short period of time to cause vaporization of the tissue. This obviously is not the case in curing an orthodontic resin, since enamel removal is not the goal.

More practical for an orthodontic application would be the effect on enamel of the argon laser at power settings and curing times, which would be used clinically. One recent study varied the laser intensity and found that argon laser bonding could reduce curing time by 75%. They reported that laser curing after regular curing resulted in greater bond strength and that a power of 200-300 mW should be used for efficient curing, and that 10 seconds was an adequate length of time for total curing of the composite.[41] Currently, the argon lasers manufactured for the purpose of composite polymerization have an energy in the range of 100-250 mW, and most cannot be adjusted to increase energy. Testing curing times and energies out of the range of in-office argon lasers would serve no purpose for the practicing clinician.

Westerman et al.[50] realized that AL treatment at low energy could considerably alter surface morphology while maintaining an intact enamel surface. The purpose of his

study was to determine the effect of AL irradiation delivered from two different systems on enamel caries-like lesion initiation and progression. The authors concluded that AL irradiation provides a certain degree of protection against *in vitro* enamel caries initiation and progression, and that it may prove beneficial in reducing the caries susceptibility of sound enamel in the clinical environment. In this study an AL was used for 10s at 231mW with a 5mm beam size, which are clinically relevant time and power settings.

Noel et al.[51] used an AL at 250 mW power for 5 s and 10 s curing durations, which are again in the range one would use clinically. In this *in vitro* study, 86 human posterior teeth were cured either with the AL (5 s or 10 s) or a CCL. The teeth were then subjected to an artificial acidic environment as a means to simulate the demineralization process intraorally. Brackets cured for 10 s with the AL showed a significantly lower lesion depth as compared to the AL for 5 s or a CCL. In a similar *in vitro study*, *Flaitz et al.*[6] evaluated demineralization resistance after argon laser irradiation, using energy levels within the range of an in office argon laser. The results of studies with a 10 second exposure at 250 mW energy level showed a 31-35% reduction in the demineralization compared with a visible light control.

A recent clinical pilot study by *Blankenau,*[5] explored the role of the argon laser on *in vivo* enamel caries development in teeth slated for extraction prior to orthodontic treatment. A split mouth design was used, with one premolar receiving AL irradiation, while the contralateral premolar served as a no treatment control.

Orthodontic bands that were modified to allow for retention of plaque against sound enamel surfaces were placed on the premolar teeth. Following a 5 week period, clinically detectable white spot lesions developed. The teeth were extracted, and the white spots were examined by polarized light microscopy. This pilot study found a mean lesion depth of 206 nm for the no treatment group compared with a mean lesion depth of 145 nm for the argon laser irradiation group. All irradiated enamel surfaces had caries with smaller lesion depths than those for their paired no treatment controls. Lesion depth decreases ranged from 23-33%. This study used a low fluence argon laser (250 mW, 5 mm beam size, 10 s). The findings of this study gives further credence to the ability of argon lasers to improve the resistance of irradiated sound enamel to *in vivo* caries development, as suggested by prior *in vitro* studies. However, this study was only for a 5 week period, and gives no indication if this demineralization resistance is permanent, or by what mechanism it occurs.

In the previous studies no mention was made as to the method of decreased caries susceptibility. *Westerman et al.*[52] showed that argon laser treatment could considerably alter the surface morphology while maintaining an intact enamel surface. It has been estimated that the effect of laser irradiation may result in lowering of the threshold pH from 5.5 to 4.78.[53] In other words, a fivefold increase in the concentration of an organic acid would be necessary to dissolve a similar amount of lased enamel compared with untreated enamel. It would be a reasonable hypothesis that this increased acid resistance is due to carbonate loss, since the loss of carbonate results in a mineral phase which more closely resembles hydroxyapatite

and is therefore less soluble than normal enamel at a give pH. Previous studies showed a similar correlation in artificial apatites with varying carbonate content.[54,55]

To summarize, it can be seen that the existing studies on AL usage at clinically relevant orthodontic time and power settings offer some theories, but no definitive answers about what causes the increased enamel resistance to demineralization. The role of carbonate is frequently speculated as a likely factor. Further *in vitro* studies, including identification of compositional, structural and physical changes in the enamel apatite effected by safe laser irradiant levels, and their correlations with subsurface demineralization and dissolution data, should permit a better understanding for optimizing conditions to achieve beneficial dissolution properties.[10]

1.2.4 Surface Scan Methods

It is not yet completely understood how and why the argon laser converts the enamel into a surface which is less prone to demineralization. Several investigators have speculated that the increased caries resistance was due to decreased solubility of the enamel resulting from an alteration in the composition of the mineral phase, in particular the carbonate concentration.[9,10,11] The opportunity for examining and understanding these changes has not been fully exploited, particularly at the fundamental level where surface science techniques can provide extensive detailed information.[17] Therefore, a review of two of the most precise surface science techniques, X-ray photoelectron spectroscopy (XPS) and time of flight secondary ion mass spectroscopy (ToF-SIMS) are appropriate.

1.2.4.1 X-ray photoelectron spectroscopy (XPS)

The basic XPS experiment is as follows. X-ray photons (hv) from a monoenergetic beam are directed onto the sample. The photons are absorbed by sample atoms with each absorption event resulting in the prompt emission of a photoelectron. Electrons from all the orbitals of the atom with a binding energy (E_b) less than the X-ray energy are excited, though not all with equal probability. Thus, some peaks are more intense in the spectra than others. Since energy is conserved, the kinetic energy (KE) of the electron plus the energy required to remove it from its orbital to the spectrometer vacuum must equal the X-ray energy. If the X-ray energy is known and the kinetic energy is measured with the electron spectrometer, the binding energy of the electron can be obtained. Due to the ultra-short sampling depth of XPS (a few nm), chemical information is acquired selectively from an ultra-thin layer at the substrate surface.[56] In practical solid state experiments, a correction for the spectrometer work function, $Ø_s$, must also be applied, normally as part of the spectrometer calibration procedure. Thus, one obtains $E_b = hv - KE + Ø_s$.

Photoelectrons of different energies are separated and counted by retardation through a focusing lens and deflection through an electrostatic analyzer onto an electron multiplier or channel plate. Although the measured binding energy (BE) is determined largely by the element and atomic orbital, it is also dependent on the chemical state and environment of the atom and can be affected by quite subtle initial and final-state relaxation effects. The technique is highly surface sensitive, owing to

the low mean free path of the photoelectrons travelling to the surface of the solid. This low mean free path also creates the need for ultra high vacuum (UHV) experimental conditions in order to allow the electrons to reach the analyzer. Electrons traveling from the sample surface towards the energy analyzer should encounter as few gas molecules as possible, otherwise they will be scattered and lost from the analysis.[57] An UHV environment has the added benefit that clean, model surfaces may be created and maintained throughout surface analysis.[17]

Since the atomic structure of each element in the periodic table (Fig. 1.6) is distinct from all the others, measurement of the positions of one or more of the electron lines allows the ready identification of an element present at a sample surface. Elements adjacent to one another in the periodic chart produce electron lines which are well separated from one another so that no ambiguity exists in identification of adjacent elements. Thus, carbon, nitrogen, oxygen and phosphorous are easily distinguishable.[58] The intensity of the signal observed is a function of the amount of material present. Each of the elements in the compound produces at least one electron line in the spectrum, with the exception of hydrogen, which is not detected by XPS.

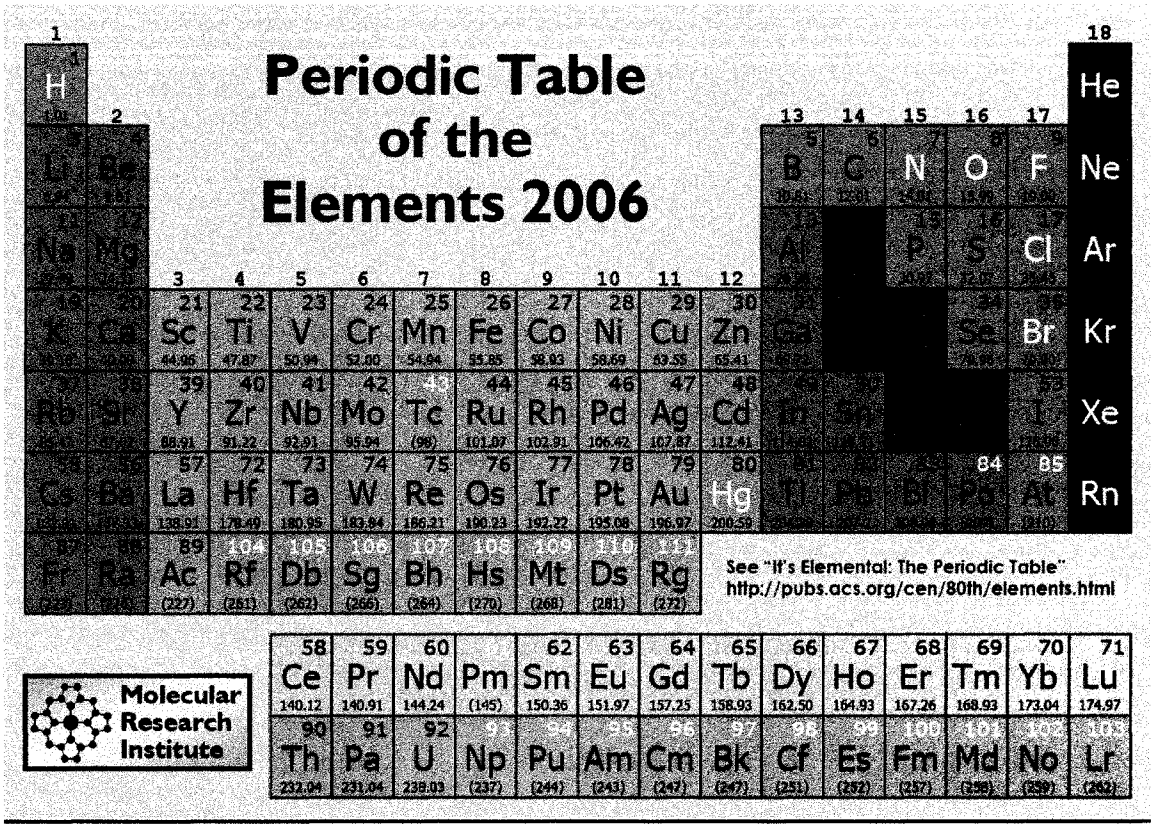

Fig. 1.6 Periodic Table (Molecular Research Institute, 2006). The mass numbers of the elements are below their abbreviations in the above chart. Thus, the mass number for C is 12.01. These are the numbers used in SIMS and XPS to differentiate atomic species. A polyatomic ion such as CO_3^{-2} is the sum of all its elemental constituents.

In the dental literature XPS has been utilized to investigate the chemical interaction of synthesized polyalkenoic acid with enamel and synthetic hydroxyapatite,[59] to monitor the adsorption of active agents from six commercially available mouthrinses,[60] to provide evidence of chemical bonding of glass-polyalkenoate cement to enamel,[61] and to examine resin-modified glass-ionomer cements, specifically to indicate that resistance to water displacement decreases as hydrophobicity increases.[62,63] *Yoshioka et al.*[63] studied adhesion/decalcification mechanisms of acid interactions with human hard tissues such as bones and teeth, and analyzed the chemical interaction of five carboxylic acids (acetic, citric, lactic, maleic, and oxalic) and two inorganic acids

(hydrochloric and nitric) with enamel and two synthetic hydroxyapatite (HAp) powders using XPS. No study was found that used XPS to investigate the changes on the enamel surface caused by AL irradiation.

However, many studies have used XPS to study the enamel surface for a variety of other reasons. Of particular interest are studies examining carbonate concentrations, which can be extracted from a detailed analysis of the C 1s region. The detailed spectrum of the C 1s region shows an asymmetric peak with a main C-C, C-H peak and weaker C-O, C=O, -COO-, and CO_3^{2-} peaks. The complete C 1s peak should be attributed to the specific composition of the enamel. Each carbon is in a different electrostatic environment and therefore exhibits a different chemical shift, producing carbon 1s peaks in different positions in the spectrum. Carbon in the most electronegative environment (CO_3^{2-}) appears at the highest binding energy. This is because the electronegative oxygen atoms withdraw electron density from the valence and bonding orbital of the carbon atom, thereby reducing the screening of the core electrons from the nuclear charge and increasing their binding energy.[64] Thus, in general, atoms with highly electronegative substituent groups can be expected to exhibit higher binding energies than the same atoms bound to groups with lower electronegativity.[58] XPS loss signals have been used as a way to extract chemical information regarding different functional organic groups (C-O, C=O, C-O-H) by looking at the loss peaks resulting from plasmons from the C 1s core level.[65]

It is the combination of high surface sensitivity and the ability to provide chemical

shift information about species observed at surfaces that is the great strength of XPS and provides its unique position among surface analysis techniques in the study of enamel.[58]

1.2.4.2 Time Of Flight Secondary Ion Mass Spectrometry (ToF-SIMS)

ToF-SIMS works by removing atoms/ions from the surface layer and then measuring the mass and quantity of the ions. Thus, it counts the number and species of the atoms per unit area of the surface, and the relative abundances can be detected. The SIMS technique has been used for more than 25 years for the analysis of dental hard tissues. This technique is very suitable for the elemental analysis of mineralized tissues, such as dental enamel, which have a high content of inorganic elements.[66] The ability to obtain compositional information from the outermost atomic layer of a solid has progressed considerably during the past number of years with the development and perfection of secondary ion mass spectrometry (SIMS). This method approaches a true surface analysis capability, since most of the compositional information originates form the outer 10 Angstroms of the surface and since the sensitivity of each of these methods is sufficient to detect a small fraction of a monolayer for most elements. As well, The technique of SIMS gives outstanding sensitivity of element detection.[67]

In SIMS, a beam of energetic ions (primary ion beam) is accelerated towards the sample. On impact, fragments of the surface are knocked-off in the process known as "sputtering". These fragments can be molecules or atoms in the ground or excited

states and positive or negative ions. It is these latter, charged particles that are detected and separated according to mass/charge ratio using a time of flight (ToF) mass spectrometer.[17] The positive or negative ions produced in this way are called "secondary ions". They can be used to characterize the elemental and isotopic composition of the sample. Most of the ejected particles originate from the uppermost layers, from a site located in the vicinity of their original position in the target.[68] The secondary ion intensities of minor matrix constituents are proportional to the atomic concentration of the constituents in the particular matrix.[58] The secondary ion beam consisting of all ionized species is separated by a mass spectrometer into individual ions on the basis of the ion's mass/charge (m/z) ratio. The use of mass separation as the analytic mode has advantages for biologic systems. First, all m/z units are recordable, from mass a mass number of 1 (hydrogen), to the mass limit of the instrument. Second, the biologically interesting elements are in this range, and isotopes can be discriminated.[69]

SIMS has proved to be outstandingly suited for charting the distributions of most elements, even at extremely low concentrations, in tooth and bone materials. In spite of this success, only a few teams, in a handful of countries, are presently engaged, to any significant extent, in conducting tooth or bone research by the application of SIMS. Of the over 400 SIMS instruments worldwide, fewer than 10 have, in recent years been reported as active in studies of tooth and bone material.[70]

The type of SIMS machine ideally suited for enamel analysis is characterized by

relatively high ion currents of the primary ion beam and hence high sensitivity, good reproducibility, and relatively good requisites for developing quantification methods for secondary ion intensities. Secondary ions are ejected with a range of different velocities, leading to different energy distributions, each reflecting the nature of the respective ionic species. Thus, monoatomic ions, such as Ca^{+2}, as a rule exhibit significantly flatter energy distributions than do polyatomic ions such as CO_3^{-2}.[70] The most familiar type of SIMS application to biological hard tissues is concentration profiling in teeth. Only recently, by aid of a "self-adjusting" electron flooding device has one also been able to record in-depth profiles in insulating specimens for negative secondary ions, such as carbonate.[70]

Quantitative SIMS of tooth and bone hard tissues, is today routinely established, and its typical artifacts, including those connected with the complicated intrinsic spectra and with the insulating nature of the specimens, have been brought under control.[67]

Both SIMS and XPS test only the outer surface of enamel, which is where potential changes from laser irradiation will occur. Based on the published data from various groups concerning the fluoride distribution and depth of laser impact in enamel, the effects of laser-fluoride can be estimated to be within 20–30μm from the enamel surface.[37] Extrapolating this data to changes in the carbonate concentration after laser irradiation shows that this depth is well within the range of both of these surface analytical techniques. No studies have used the modern surface science techniques of XPS and Tof-SIMS combined with AL irradiation levels used clinically in

orthodontics, to study carbonate changes in the enamel surface.

1.2.5 Laser Safety

A major clinical concern with laser irradiation of teeth is the potential for overheating the tooth pulp, which could lead to pulpal necrosis.[71] For temperature change at the surface of a tooth, the duration of time over which the energy is delivered is equal in importance to the amount of energy delivered. A few *in vitro* and *in vivo* studies have suggested the safeness of irradiation conditions used in orthodontics.

Enamel and dentine are the parts of the tooth that have been studied most using surface sensitive techniques. Although the remaining discussion will refer almost exclusively to these materials, it is worth mentioning the anatomy of the rest of the tooth. The innermost region is known as the pulp.[17] The outer region of the pulp contains the odontoblastic cell bodies, which extend a short distance into the next layer, which is dentine. The dentine is then covered by enamel. The bulk of the pulp is similar in composition to connective tissue, containing various types of cells, collagen fibres, nerve trunks, lymphatics and blood vessels. It serves to provide metabolic support for the odontoblast cells and dentine.[17] Thus, when treating the enamel of the tooth, it is important that a laser not damage the enamel, or the underlying dental tissues, which are often more sensitive.

One of the greatest concerns regarding laser application in dentistry is the temperature change in the dental tissues under irradiation. Special attention has been paid to pulp tissue. More specifically, it has been shown that 15% of teeth submitted to 5.5 ^{o}C in

temperature variation developed an irreversible inflammation condition.[71] *Kurachi et al.*[72] observed that the conventional curing light induced a temperature variation similar to the one caused by the argon laser at 300 mW of power. After 45 seconds of AL exposure, the temperature variation in the pulp chamber was above 5 °C, a situation that is critical for pulp tissue. When they decreased the laser power to 200 mW, the temperature rose above 5 °C only after 150 seconds. The overall results showed that for the recommended AL laser curing times in orthodontics (10 s), the temperature increases were 3 °C or less, which can be considered safe. *Powell et al.* [73] studied the pulpal temperature changes induced by an AL and a CCL under common conditions. In this study, the results showed that for the recommended laser curing times the temperature increases were 3 °F or less. The authors also demonstrated that the *in vitro* increases of the pulp chamber temperatures, when laser light is used in the common curing times, were significantly lower than equivalent times for the CCL. In both cases a continuous increase in temperature was observed for longer times of exposure. This is in agreement with another study which found that if the power is less than 500 mW and exposure time 10 s or less, little pulpal effect is seen from the AL.[40]

Pulpal histology from *in vivo* testing confirms that the argon laser, when used at the energy levels recommended for restorative dentistry applications, creates neither short-term nor long term pulpal pathology.[74] The literature clearly shows, that under the conditions the AL is used in orthodontics, there should be no danger to tooth structure.

When used in orthodontics, the laser is not only exposed to tooth structure. There will also be some exposure to surrounding soft tissues. *Brenneise and Blankenau* [75] found that clinically relevant argon laser exposure (10 s), of parakeratinized gingival adjacent to teeth undergoing restoration does not cause lasting damage. Tissues exposed for 30 s did show signs of hyalinization and necrosis that was still not resolved at 5 days. The power output in this study was 231 mW with a 5mm spot size for 10, 20, or 30 seconds. During use as a polymerization instrument, the argon laser would never intentionally be applied to the adjacent soft tissues. However, due to the proximity of orthodontic brackets to the gingival tissues, exposure will nonetheless occur. The above study seems to indicate, that if this exposure is 10 s or less, there should be no long-term problem.

Argon laser light is in the blue-green range so the operator and patient's eyes must be protected, since exposure to this high energy light could lead to visual damage. Laser loses no power over distance, as in the CCL. Therefore it is imperative that the operator and patient both have eye protection, which totally filters light from the UV spectrum.

1.2.6 Summary

Use of the argon laser in orthodontic practice has yet to gain widespread clinical acceptance. Although the AL definitely has its advantages over CCL's, such as reduced curing times and no loss of power at greater distances; it is far more expensive and takes up considerably more space than conventional curing lights. As

it stands now, it would seem more benefits of the AL need to be uncovered for it to gain a wider acceptance by practicing clinicians.

XPS and ToF-SIMS analysis of AL irradiated enamel can provide a detailed insight into the carbonate content of the enamel before and after AL irradiation. An understanding of the elemental changes occurring on the enamel surface is necessary to thoroughly understand and convey the possible advantages of the AL.

1.3 Statement of the Problem

Orthodontic treatment often takes upwards of two years. During this time the orthodontic appliances can act as reservoirs for plaque, which can subsequently cause demineralization of the enamel surface. The AL is one technique which can be used to cure the resin material attaching orthodontic brackets to the teeth. One of the main advantages of the AL is a potential for increased enamel resistance to demineralization. However, the question remains as to why the enamel surface is more resistant to demineralization. This study will use modern surface science techniques to determine what effect AL irradiation has on the chemical, and particularly the carbonate composition of enamel.

Carbonate has profound effects on both the physical structure and chemical stability of dental enamel. If it can be shown that there is a reduction in the concentration of carbonate in enamel after exposure to AL irradiation, this may offer an important explanation as to the increased demineralization resistance. An elemental change

such as this could be considered permanent, bringing with it a permanent increased enamel resistance to demineralization. This would be an important addition to the orthodontist's armamentarium, as current techniques to reduce cavities are not permanent or require patient compliance.

1.4 Research Questions

1. Is there a difference in the carbonate concentration of the enamel surface after exposure to argon laser irradiation (10 s) as tested by XPS?
2. Is there a difference in the carbonate ionic counts of the enamel surface after exposure to argon laser irradiation (10 s) as tested by ToF-SIMS?

Secondary objectives of this study are to analyze the difference in the carbonate concentration of the enamel after argon laser irradiation for 120 seconds. When collecting data for the carbonate concentrations with either XPS or ToF-SIMS, a range of other chemical data becomes available. This data provides information in regards to concentrations of other enamel components. These findings will also be analyzed and commented on, but will not be a primary focus of the study.

1.5 Null Hypotheses

1. There is no difference in the carbonate concentration of the enamel surface after exposure to argon laser irradiation (10 s) as tested by XPS.
2. There is no difference in the carbonate ionic counts of the enamel surface after exposure to argon laser irradiation (10 s) as tested by ToF-SIMS.

Chapter Two: Research Paper

2.1 Introduction

Dental caries is the most common chronic disease in children and adolescents in the United States, as 85% of 17 year-old individuals have developed dental caries.[30] This problem can often be accentuated in patients undergoing orthodontic treatment. Often the first sign of caries is the presence of areas of demineralization, which are also known as "white spot lesions". Orthodontic treatment requires the bonding of appliances to the teeth, and treatment can often take upwards of two years. During this time the orthodontic appliances can act as reservoirs for plaque, which can subsequently cause demineralization of the enamel surface. This demineralization is caused by the acids excreted by bacteria as a product of their metabolism of carbohydrates from the plaque.[17]

Composite resins are used to bond the orthodontic brackets to the teeth. For years the standard process has been to use a conventional curing light (CCL), which hardens the composite resin and fixes the bracket to the tooth. The argon laser is a recently developed technique which can also be used to cure this resin material. One of the main advantages of the AL is a potential for increased enamel resistance to demineralization.[4-8,40,41,50,51] However, the question remains as to why the enamel surface is more resistant to demineralization after AL irradiation. Several investigators have speculated that the increased caries resistance was due to decreased solubility of the enamel resulting from an alteration in the composition of the mineral phase,[9-11]and in particular an alteration in the concentration of carbonate.[24] One theory is based on the fact that bound carbonate is released when dental enamel is

heated or irraditated.[44]

Enamel is the hardest biological substance in the human body and is a crystalline structured material consisting of both a mineral and an organic phase. The mineral phase predominates (96wt.%) and consists primarily of calcium hydroxyapatite $(Ca_5(PO_4)_3(OH))_2$. The remainder of the enamel is made up of 3% water and 1% organic matter including proteins and lipids. Pure hydroxyapatite (HA) does not occur on a macroscopic scale in biological systems. Enamel, dentine, cementum and bone are instead made up from a calcium-deficient and carbonate-containing apatite analogue.[18] The primary substitution in carbonated apatite as opposed to hydroxyapatite, is the carbonate (CO_3^{2-}) replacing the phosphate (PO_4^{3-}) group. Planar CO_3^{2-} substitutes for tetrahedral PO_4^{3-}, which disrupts the crystal structure and weakens chemical bonds, leading to a more acid soluble mineral.[17] At a given pH, the solubility of hydroxyapatite is approximately an order of magnitude below that of carbonated apatite,[37] meaning that demineralization will occur much easier.

Carbonate has profound effects on both the physical structure and chemical stability of dental enamel. If it can be shown that there is a reduction in the concentration of carbonate in enamel after exposure to AL irradiation, this may offer an important explanation as to the increased demineralization resistance. An elemental change such as this could be considered permanent, bringing with it a permanent increased enamel resistance to demineralization. This would be an important addition to the orthodontist's armamentarium, as current techniques to reduce cavities are not

permanent or require patient compliance.

This study used modern surface science techniques to determine what effect AL irradiation had on the chemical, and particularly the carbonate composition of enamel. The opportunity for examining and understanding these changes has not been fully exploited, particularly at the fundamental level where surface science techniques can provide extensive detailed information.[17] Two of the most precise surface science techniques, X-ray photoelectron spectroscopy (XPS) and time of flight secondary ion mass spectroscopy (ToF-SIMS) were used to assess carbonate changes in the enamel surface after AL irradiation. Both of these techniques are very suitable for the analysis of mineralized tissues such as enamel.[58,66]

XPS and ToF-SIMS analysis of AL irradiated enamel can provide a detailed insight into the carbonate content of the enamel before and after AL irradiation. In view of the profound effect of carbonate on both the physical structure and chemical stability of dental enamel, an understanding of the elemental changes occurring on the enamel surface is necessary to thoroughly understand and convey the possible advantages of the AL.

2.2 Methods and Materials

2.2.1 Sample Selection and Handling

11 Mandibular 3rd molar teeth previously scheduled for extraction were collected. No previous study using these methods was found therefore sample size could not be

calculated *a priori*, so an arbitrary sample size of 11 was arrived at, taking into account the time and cost restrictions of using the surface analysis equipment required. The teeth collected for use in this study were extracted only when patient care indicated a need for extraction and were not labeled at any time with the patients' name, so that the confidentiality of the patients could be protected. Therefore, the procedures used for collecting teeth fell under the expedited review category of the Health Research Ethics Board of the University of Alberta (Appendix A). As well, informed consent was obtained from the patient's for the use of their teeth in an experimental study (Appendix E).

11 human mandibular 3rd molar teeth, which were recognized as macroscopically non-carious, free of developmental disturbances, and with no damage to the enamel surface were selected. After selection the surface of the teeth were wiped clean, such that there was an absence of cellular debris on the surface of the teeth. After extraction, the teeth were stored separately in 0.1% thymol solution (one jar per tooth). Storage solution was used to prevent dehydration of teeth collected immediately after extraction and to prevent bacterial and fungal growth in the storage media. The chemical nature of the storing agent may affect the tooth structure and material properties at the tested interface. *Ziskind et al.*[76] evaluated the use of 0.1% cetylpyridinium chloride (CPC) as a new storage solution and to assess the possible effect of 0.1% thymol on microleakage and bond strength. Forty extracted human teeth were collected from 10 different dental clinics. Immediately after extraction, the teeth were randomly divided and immersed in four different storage solutions. Two

test solutions of 0.1% CPC and 0.1% thymol were compared with phosphate-buffered saline and to 3% H_2O_2. Bond strength test and dye penetration evaluation were then carried out. The findings suggested that the use of 0.1% CPC as storage solution does not affect bond strength to enamel. Therefore, thymol seemed a suitable storage media for this study, since it should have minimal impact on surface characteristics, and will provide an additional antibacterial component.

After thymol storage for a minimum of 7 days the teeth were then rinsed with distilled water. The tooth crown was separated from the root and sliced longitudinally (disto-mesial), and the buccal portions of the different crowns were separated from the rest of the tooth. The buccal portion of the tooth was further sectioned into two samples for each of the 11 teeth. These samples were reduced in size to approximately 2 mm x 5 mm. The cutting was performed using a diamond disc (Brasseler Dental Instrumentation, Savannah, Georgia, USA). The dentinal layer was completely removed from the sections with a football shaped diamond bur (Brasseler Dental Instrumentation, Savannah, Georgia, USA). Since each tooth had been sectioned in two pieces one section received AL irradiation, while the other section acted as a control. The tooth section receiving the AL irradiation had a notch placed in one corner with a diamond disc, while the control group had no such marking (Fig. 2.1). Thus, the irradiated surface enamel regions (experimental) and adjacent sound surface enamel (control) were analyzed from the same tooth surface. Only the principle investigator knew which section of the tooth was exposed, the technician and statistician doing the surface testing did not have this knowledge.

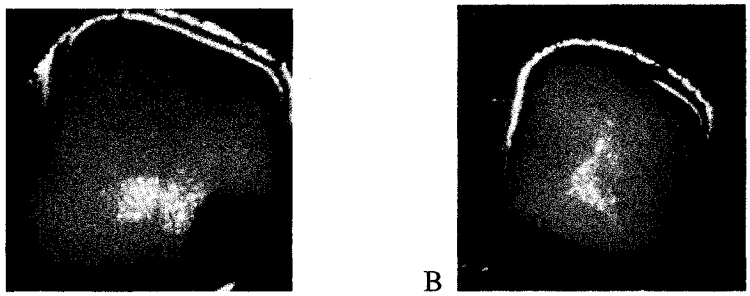

Fig. 2.1 Paired tooth samples. A- experimental; B- control

Mandibular 3rd molar teeth were chosen for two reasons. These teeth are easily available due to the high frequency of their extraction. Also, lower molar teeth are a difficult area for orthodontic patients to clean. Therefore, enamel changes on lower molar teeth are of particular interest. The buccal surface was used because this is the surface where orthodontic brackets are placed.

2.2.2 AL Irradiation

An AL (AccuCure 1000, LaserMed, Salt Lake City, UT) was used in this study (Appendix C). This AL is classified as a 3b laser. Safety approval was obtained from the U of A safety officer (CS) after a radiation protection lecture was given to the primary investigator (Appendices B and D). The AL output was tested with a Curing Radiometer (Model 100, P/N 10503, Demetron Research Corporation, Danbury, CT) on a regular basis to ensure that the chosen output value (AL: 250 mW/cm^2) was the actual output value throughout the study. An AL leakage current test was performed by a specialized technician (CS) at the University of Alberta. The readings of the AL leakage currents were taken with a BK Precision Model 1655 AC Power Supply and leakage Tester U of A # 162679. The AL was well under 500 micro amp hospital

standards.

It is very difficult to compare the numerous reports of the effects of laser irradiation on human enamel because of the variety of lasers used, their differing energy densities, laser line wavelengths and variations in laser interaction time.[77] This study attempted to mimic the conditions in which the AL would be used in an orthodontic setting. An exposure time of 10 seconds was chosen,[41] a power setting of 250 mW, and a 5 mm beam size. This time and power setting has been shown to be clinically safe and effective.[78] The AL source was held 3-5 mm from the irradiated samples, which would be similar to the distance clinically. After the AL exposure the teeth were stored in distilled water for less than 7 days, until the appropriate surface testing was performed.

As an additional corollary to this study, after surface testing was performed on the irradiated samples, they were then further exposed to AL irradiation for another 110 seconds, for a total of 120 seconds. Further surface testing was then done on the experimental sections, to record changes this increased time of irradiation produced. This additional irradiation was performed to exaggerate potential changes in the chemical composition of the enamel mineral, which may occur with a curing time outside of clinical norms.

2.2.3 Surface Testing

The composition near the surface is very likely to be different from the bulk of the enamel. Therefore it is important to use techniques that test only the surface of the

tooth. For this reason the surface sensitive analytical tools (XPS and SIMS) were used to study the enamel surface before and after AL irradiation, and assess any differences in the mineral composition. In particular, the carbonate content of the enamel in the outer layer of the enamel was measured. The choice of carbonate for analysis in this study was based on the assumption that it might display a change in concentration following AL irradiation, and contribute to increase caries resistance of AL irradiated enamel.

Surface testing was done on the 2 sections from all 11 teeth. This permitted a direct comparison of the effects of the AL on the mineral content of the enamel, because comparisons were from the same tooth, surface (buccal), and the same patient. SIMS and XPS were performed on all the samples, and comparisons were made before and after AL irradiation for 10 seconds. After re-exposure for a further 110 seconds, the irradiated teeth were again analyzed with XPS and SIMS.

Surface analysis is the use of microscopic chemical and physical probes that give information about the surface region of a sample. In this case the analysis is done to provide information about the concentration of carbonate in the enamel.

2.2.3.1 X-Ray photoelectron spectroscopy (XPS)

One tool used to measure the carbonate content of surface enamel was X-ray photoelectron spectroscopy (XPS) (Fig. 2.2), which is also known as electron spectroscopy for chemical analysis (ESCA). XPS uses X-rays to produce

photoelectrons from the surface layers of atoms in a solid sample. The emitted electrons are analyzed according to their kinetic energy and the spectrum so produced is used to identify the elements present and their chemical states. XPS is a highly selective and specific method of surface analysis.[79] The method allows the upper 1 to 10 atomic layers (0.5 to 5 nm) to be investigated. In attacking problems XPS can provide data about the elemental composition of a surface, determine the chemical states of the surface atoms (e.g. degree of oxidation), and can be used to determine the molecular structure of the surface (e.g. type and relative abundance of functional groups associated with species adsorbed at the surface). As well, XPS is a relatively non destructive technique compared with other methods of surface analysis. No surface species are removed during XPS measurements, and the soft X-ray source used for excitation avoids many of the problems associated with thermal degradation of sensitive materials. Sample composition remains constant during analysis.[58]

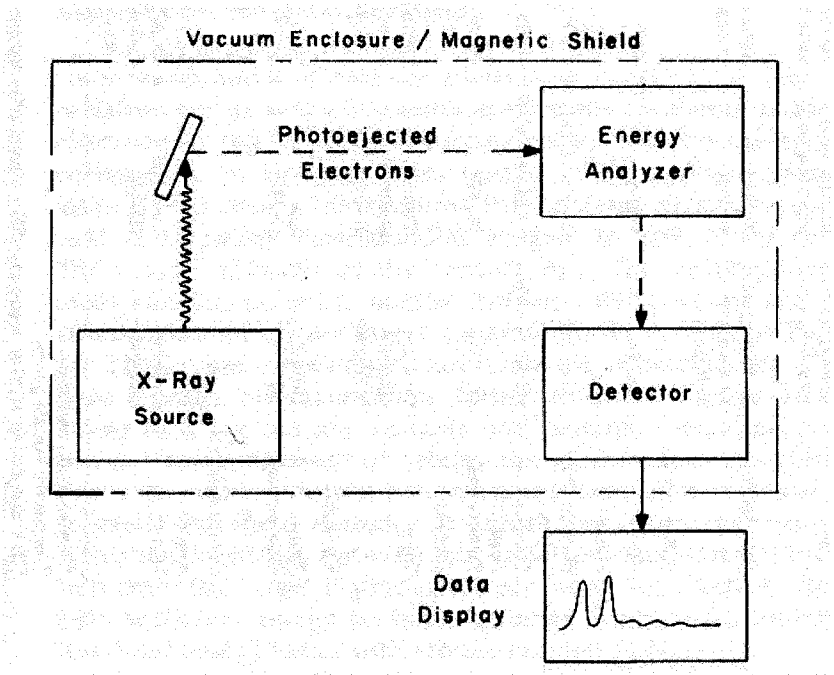

Fig. 2.2 The basic elements of an X-ray photoelectron spectrometer. The function of the spectrometer are to produce intense X radiation, to irradiate the sample to photoeject core electrons, to introduce the ejected electrons into an energy analyzer, to detect the energy-analyzed electrons, and to provide a suitable output of signal intensity as a function of electron binding energy (Riggs WM, Parker MJ. Surface Analysis by X-ray Photoelectron Spectroscopy. Book: Methods of Surface Analysis. Elsevier Scientific Publishing Company, 1975: 123).

In XPS, the sample is irradiated with soft X-ray photons. The X-ray excitation of the inner shell electrons of target atoms induces direct emission of photoelectrons from the sample surface. The energy of photoelectrons is characteristic of the target material, and measurement of the energy spectrum (number count vs. kinetic/binding energy) provides valuable information about the top 2-20 atomic layers, depending on the material studied. Peak position and peak area are used to evaluate the composition, while the peak shape gives unique information about the chemical shifts or chemical bonds of the elements.[1] Of particular interest to this study is the ability for XPS to differentiate between various chemical bonds in the carbon spectrum, and thus distinguish the different chemical states of carbon present in enamel.

The electron binding energies, as measured by a high resolution electron spectrometer, are used to identify the elements present and to provide information about the valence states or chemical bonding environments of the elements thus detected. The depth of the analysis, typically the outer 3 nm of the sample, is determined by the escape depth of the photoelectrons and the angle of the sample plane relative to the spectrometer.

The x-ray beam is pointed towards the enamel surface region to be analyzed and is utilized to excite electrons from the core-level orbitals of the atoms. The kinetic energies (E_K) of the emitted photoelectrons are characteristic of the element and atomic orbital from which they originate, being related to the binding energy (E_B) through the relationship: $E_B = hv - E_K$ (Fig. 2.3)[1]. The electrons are detected and counted according to the energy they possess. An electron analyzer then collects the

emitted electrons and separates them based on their intitial binding energy before leaving the surface, and formulates a spectrum.[58] Each binding energy matches a specific atom type (e.g. 284.6 eV matches carbon). Each peak area is proportional to the number of atoms being present in the studied element. The spectrum gives an overall picture of the counts for a variety of elements. This spectrum serves as a survey tool to determine the areas of interest which are further examined with high resolution scans. Identification of chemical states often can be made from exact measurements of peak positions and separations, as well as from certain spectral features.[1] In this study, a further high resolution spectrum was acquired for carbon, and subsequent data processing provided any changes in the carbonate quantity.

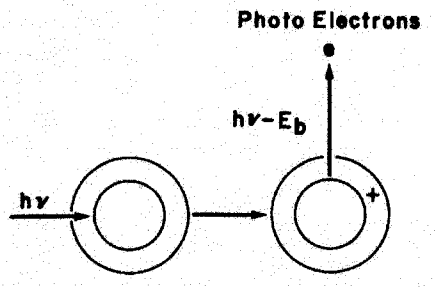

Fig. 2.3 X-ray Absorption. X-ray photons (hv) from a nearly monoenergetic beam are directed onto the sample. The photons are absorbed by sample atoms with each absorption event resulting in the prompt emission of an electron. Electrons from all orbitals of the atom with a binding energy (Eb) less than the x-ray energy are excited, though not with equal probability. Thus, some peaks are more intense in the spectra than others. Since energy is conserved, the kinetic energy (KE) of the electron plus the energy required to remove it from its orbital to the spectrometer vacuum must equal the X-ray energy. If the X-ray energy is known and the kinetic energy is measured with the electron spectrometer, the binding energy of the electron in the atomic orbital can be obtained: Eb = hv – KE. (Riggs WM, Parker MJ. Surface Analysis by X-ray Photoelectron Spectroscopy. Book: Methods of Surface Analysis. Elsevier Scientific Publishing Company, 1975: 107).

The basic elements of an X-ray photoelectron spectrometer consists of the X-ray source, the energy analyzer, the detector and the data display (Fig. 2.2). True source monochromatization can be achieved through crystal dispersion of the generated X-ray (Fig. 2.4). For the purpose of producing a photoelectron spectrum, the X-ray source will ideally produce monochromatic radiation having sufficient energy to excite core electrons of all the elements in the periodic table. A monochromatic source is desirable because the width of the incident X-ray line contributes to the width of the resultant photoelectron line; the width of the photoelectron peaks in turn affect the resolution of the electron spectrometer, that is, the ability of the system to distinguish between closely spaced peaks. X-ray sources are constructed in a variety of ways, but they share basic elements. The filament is heated by means of a current which produces a high density electron cloud around the filament due to the thermionic emission. In such cases, the spectra are charge corrected to C 1s hydrocarbon component at 285.0 eV binding energy. Modern XPS machines operate with a charge neutralizer, which is important when analyzing insulating materials, of which enamel is one. After bombardment with electrons, a charge can begin to build up on the enamel surface, which will distort measurements. The charge neutralizer greatly reduces this distortion.

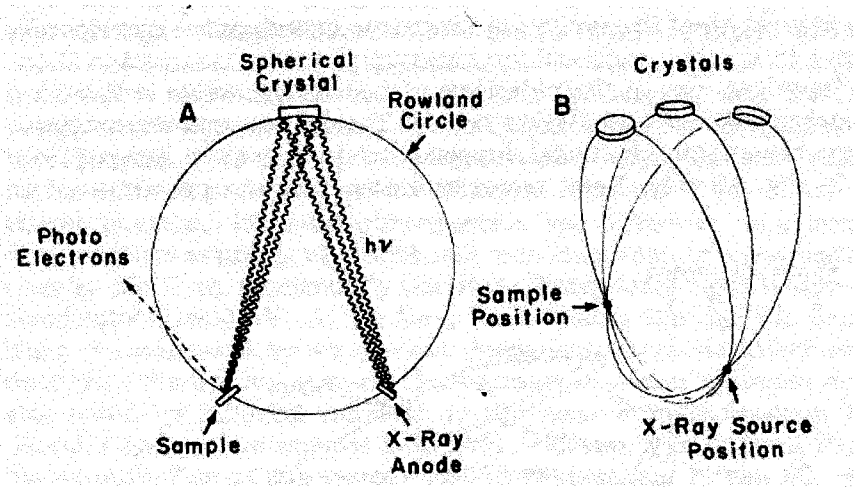

Fig. 2.4 Monochromatizing the X-ray source. (a) The basic elements of a crystal monochomator; (b) the three-crystal geometry utilized in a commercial application of this technique. One of the objectives of instrument design is to effectively monochomatize the excitiation source. If its energy width can be reduced, a corresponding reduction in the line width of the resulting photoelectron peak will be obtained (Riggs WM, Parker MJ. Surface Analysis by X-ray Photoelectron Spectroscopy. Book: Methods of Surface Analysis. Elsevier Scientific Publishing Company, 1975: 128).

The central instrumental element of the XPS techniques is the energy analyzer. The function of an energy analyzer is to measure the number of photoejected electrons as a function of their energy. The analyzer must be placed in a suitable vacuum environment, with pressure less than about 10^{-5} torr to minimize electron scattering through collision with residual gas molecules. In this case electrons traveling from the enamel surface towards the energy analyzer should encounter as few gas molecules as possible, otherwise they will be scattered and lost from the analysis.[57] To eliminate this phenomenon an ultra high vacuum (UHV) is needed. The Axis-Ultra XPS used in the present study operated under an ultra high vacuum. The UHV also serves to minimize the influence of stray magnetic fields, which can also impact electron analysis, and it is necessary to cancel these fields within the analyzer to

minimize the effects on the electron movements. The vacuum chamber also reduces the partial pressure of residual gases to a level that will not contaminate the sample surface and interfere with the study of that surface. XPS has a sampling depth of only a few atom layers and is therefore highly surface specific. When taken with elemental sensitivities of the order of 0.3 % of an atom layer, this surface specificity implies that the techniques are sensitive to surface contamination. The UHV can help to keep contamination levels low and under the acceptable rate of contamination (no more than 0.05 atom layers in 30 min).[57]

The XPS scans were acquired using an AXIS ULTRA XPS (Kratos Analytical, Manchester, UK, Fig. 2.5), which employed monochromatic AlKα x-rays (hv = 15 eV), as well as charge neutralization. The x-ray gun was operated at 210 W. The sample was placed relative to the analyzer to give a 90 degree takeoff angle, where the takeoff angle is defined as the angle between the surface normal and the axis of the analyzer lens. This allowed analysis of the elements in the top few atomic layers of the enamel surface. All samples had a survey spectrum (0-1000 keV), as well as high resolution spectra of the elements oxygen, carbon, calcium, phosphorus, and nitrogen performed. The survey scan was performed with a pass energy of 160 eV, while 20 eV was used for the high resolution spectra. Data acquisition and analysis was performed by Kratos XPS Casa software on a Sun computer system. The samples were loaded via a glove box to provide a controlled UHV environment (Fig. 2.6). The specimens were allowed to degas under high vacuum (10^{-10} torr) for at least 48 hours and were then exposed to the incident AlKα beam for alignment and signal

optimization for approximately 1 minute prior to analysis. Survey scans and high resolution scans of the the C 1s, Ca 2p, O 1s, N 1s and P 2p regions were recorded. The high resolution spectra are labeled with the abbreviated elemental name, followed by the quantum number of the ejected photoelectron. The C 1s spectrum was used to analyze the percentage of carbon present as carbonate. Four chemical states of carbon were fitted to each C 1s peak envelope. The components are C-C/C-H at 285eV. The next carbon species corresponds to ether carbon C-O, followed by CO_2^- and carbonate carbon (CO_3^{-2}) has the highest binding energy in the spectrum.

Fig. 2.5 AXIS ULTRA XPS (University of Alberta, Surface Sciences)

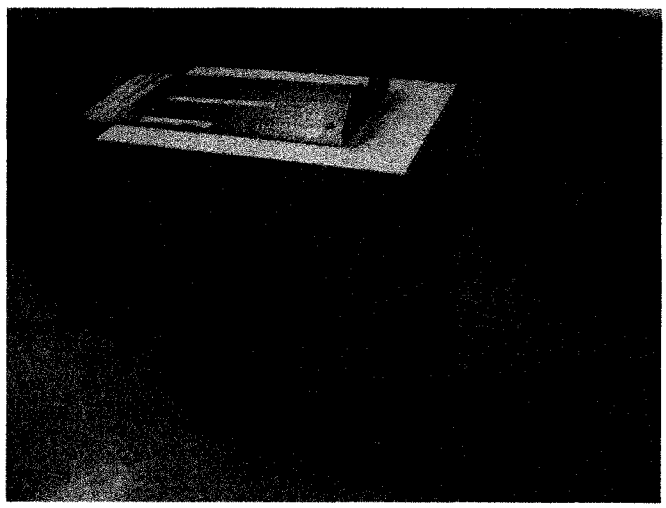

Fig 2.6 Ultra High Vacuum Transfer facility (University of Alberta, Surface Sciences)

The analysis of the spectra was performed via:

- Peak shape and background method: Following a Shirley-type background subtraction, the individual photoemission features are fitted with representative Gaussian distributions using least-squares optimization. The peak positions, amplitudes, and full width at half maximum parameters are obtained from the Gaussian distribution analysis. The peak areas correspond to the area with respect to the background subtraction.
- Quantization method: The atomic concentrations are calculated using the algorithm and sensitivity factors contained in Kratos Analytical Software.

SUMMARY- It is possible to investigate directly the chemical composition of a surface and determine the chemical differences, if any, of different surfaces. The XPS analysis was used to determine any differences in the carbonate concentration of enamel, before and after AL irradiation.

2.2.3.2 Time Of Flight Secondary Ion Mass Spectrometry (ToF-SIMS)

A number of problems arise in the analysis of the inorganic elements of dental hard tissues in small, well-defined morphological areas. The hardness of enamel and the isolating properties of the hard tissues make the use of various probe techniques difficult. Further, many of the inorganic elements have such low concentrations that few methods are appropriate for their analysis. Secondary ion mass spectrometry has proven to be a useful method.[70,66]

Time of flight secondary ion mass spectrometry (ToF-SIMS) is based on the fact that ions with the same energy but different masses travel with different velocities. Basically, ions formed by a short ionization event are accelerated by an electrostatic field to a common energy and travel over a drift path to the detector. The lighter ones arrive before the heavier ones and a mass spectrum is recorded. Measuring the flight time for each ion allows the determination of its mass.

To begin the SIMS analysis, a solid surface is bombarded by primary ions of a given keV energy. The primary ion energy is transferred to target atoms via atomic collisions and a so-called collision cascade is generated. Part of the energy is transported back to the surface allowing surface atoms and molecular compounds to overcome the surface binding energy. SIMS is destructive in nature because particles are removed from the surface. The primary ions gradually erode the surface layer of the specimen, in the form of atoms, molecules or larger clusters, neutral or ionized. In order to receive chemical information on the original undamaged surface, the primary ion dose density must be kept low enough ($< 10^{13}$ cm^{-2}) to prevent a surface area from being hit more than once. This so-called static SIMS mode is widely used for the characterization of molecular surfaces.

The removal of secondary ions from the sample surface by the primary ion beam is known as sputtering (Fig. 2.7). In SIMS applications, extremely short pulses of the ion gun (duration below 1 nanosecond) repeatedly strike the target. The ions produced by the ion gun are the primary ions, and the ions which escape the sample

after the ion pulse are the secondary ions, which can be positive or negative. The secondary ions generated by each primary ion pulse are accelerated to a constant kinetic energy, and then fly through a field free space before they hit a detector, where their intensity is measured as a function of flight time. The positively or negatively charged secondary ions are extracted and deflected successively in an electrostatic and a magnetic field, and emerge mass separated as well as energy focused. They are finally collected, for the quantification of total arrival rate. During the drift time of the secondary ions, the extraction field can be switched off and low energy electrons can compensate for any surface charging caused by primary or secondary particles (charge compensation). Thus, insulators such as enamel can be analyzed without any problems. Prior to charge compensation, insulators were difficult to analyze, due to the fact that ions from the primary ion beam would charge the surface and distort the measurements.

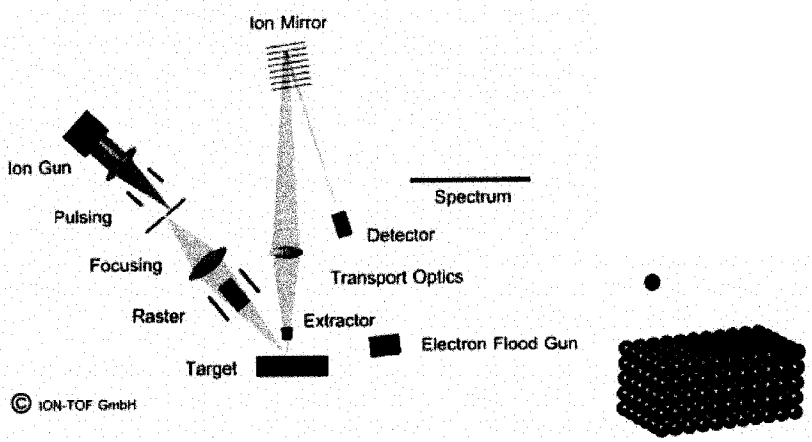

Fig 2.7 Diagramatic representation of SIMS procedure. The figure on the right is a simulated atomic closeup of the target, and is a representation of primary ion beam about to interact with sample surface. This primary ion will "sputter" off secondary ions from the surface which will enter the spectrometer to be measured. (ION-TOF, Germany).

The TOF mass spectrometer is based on the simple fact that ions with the same energy but different masses, travel with different velocities. Since ions with different masses have different velocities at a given kinetic energy, the measured flight times of the sputtered secondary ions can easily be converted to their elemental mass numbers (Table 2.1), and organized into a spectrum (Fig. 2.8). This secondary ion mass spectrum gives information about the elemental and chemical composition of the material. Ion currents may then be recorded as functions of mass number, and stored in the computer. SIMS gives us relative ion counts. The ionization rates are a function of an elements position in the periodic table, as well as their charge. Although SIMS is not considered to be a truly quantitative technique, a certain level of quantification is possible through ion intensity ratios. Therefore, a peak area ratio analysis in the negative spectrum can be performed on the ion m/z 60 so as to gain a quantitative measure of the carbonate levels in the enamel.

Mass Number	Positive Spectra	Negative Spectra
12		Carbon
14		Nitrogen
16		Oxygen
31	Phosphorous	
40	Calcium	
60		Carbonate(CO_3)
95		Phosphate (PO_4)

Table 2.1 Mass numbers for various elements and compounds

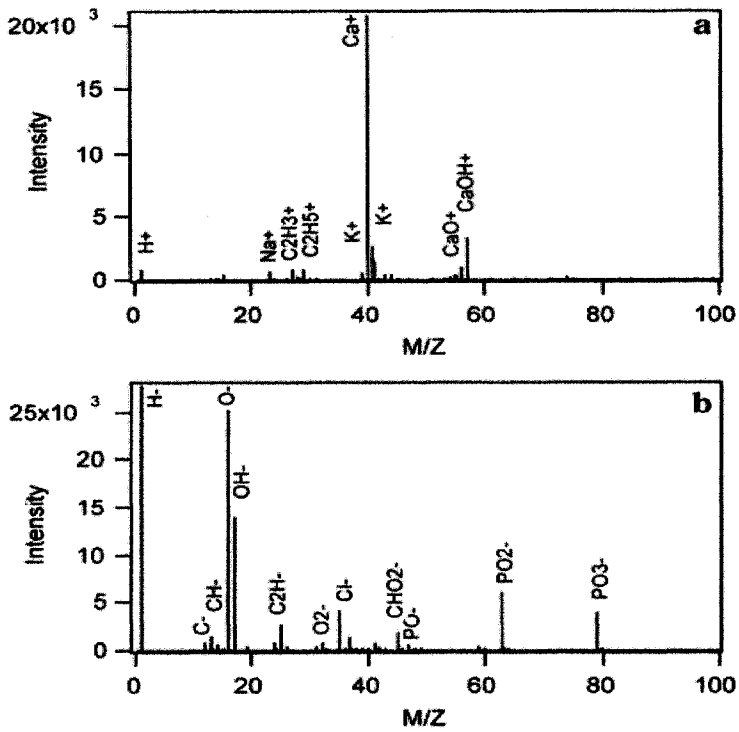

Fig. 2.8 Sample SIMS spectra for positive spectra(top) and negative spectra (bottom). [80]

As in XPS, the SIMS analysis is performed in an ultra high vacuum environment. The arrival rate at the sample surface of gaseous species from the vacuum environment can exceed the arrival rate of incoming ions. SIMS experiments operating under these conditions are usually performed in an UHV environment to avoid these complications.

The SIMS used in the current study employed a liquid source gallium ion beam, which was scanned across the sample surface, and resulted in the emission of secondary electrons and atoms. These secondary particles originated within, and consequently carried information about, the most superficial 1-2 nm of the samples. The particles are collected and quantified to separate the ions according to their mass/charge ratio. The individual ions in this study were detected in one of two ways: mass spectrum, which is the total composition of the secondary ion beam, and ion counting, which is a quantitative analysis.

Analysis was performed using the ION-TOF IV instrument (ION-TOF, Germany, Fig. 2.9). The samples were transferred in situ from the AXIS ULTRA XPS into the ToF-SIMS IV, which minimized any surface contamination. A 25 keV Ga^+ primary ion beam about 50 μm in diameter and 3.2 pA in current was used at an incident angle of 45^o to surface normal. The secondary ions were accelerated to ± 5 keV, extracted into an electrostatic field and then travelled over a drift path to the detector. Measuring the flight time for each ion allows the determination of its mass. Charge compensation was achieved by using a pulsed low energy electron source. Data were

acquired over a mass range from m/z = 0 to 1000 for both positive and negative secondary ions. The ion beam was moved to a new spot on the sample for each spectrum. The total ion dose used to acquire each spectrum was less than 2×10^{12} $ions^{cm-2}$. Such a dose lies well below the damage threshold value of 1×10^{13} $ions^{cm-2}$ for static SIMS. The area of analysis for each spectrum was 100μm x 100μm. A Sun computer system with TOF IV SIMS software (ION-TOF, Gmbh) was used for spectral acquisition, storage and data processing. The recorded mass spectra were analyzed to provide elemental intensity data. The intensity of carbonate (CO_3^{-2}; 60.01 mass number) was normalized against the intensity of the Ca^{40} ion (40.078 mass number), to eliminate the influence of intensity fluctuation caused by inherent variations in mineral density of enamel. In each sample, a ratio of carbonate/ Ca^{40} values was obtained to represent the change of the concentration of carbonate from the control to the experimental section. As well a ratio of carbonate to phosphate (PO_3^{-2}, mass number 94.971) was calculated and comparisons again made between the control and experimental sections. This was done to eliminate systematic differences in the absolute intensities observed for the positive and negative spectra and false influence from possible contamination due to different synthesis and sample preparation processes.[80]

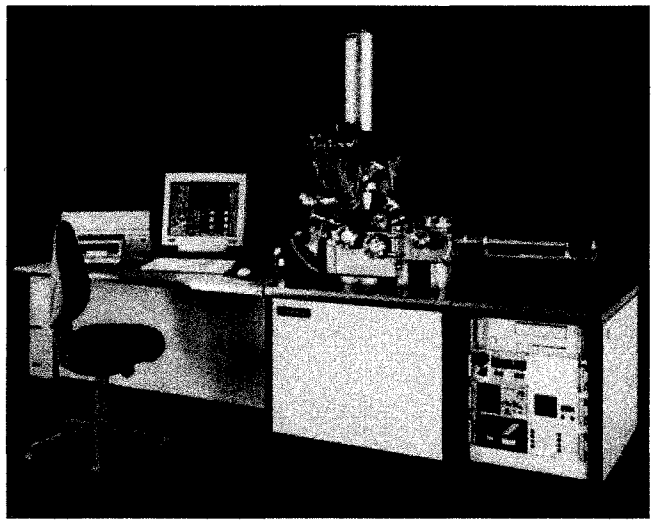

Fig. 2.9 TOF-SIMS IV (ION-TOF, Germany)

2.3 Results and Discussion

2.3.1 X-Ray photoelectron spectroscopy (XPS)

A typical XPS survey spectrum from the control and experimental sections of enamel is shown in Fig. 2.10. The peaks are labeled with the abbreviated elemental name, followed by the quantum number of the ejected photoelectron. Thus, the main peaks present are labeled as Ca (2s and 2p), P 2s, O 1s, C 1s and N 1s, with other smaller peaks present as well, which were not considered in this study. The area under the peaks allows quantification of the relative amounts of each element present, while the shape and position of the peaks represent the chemical state of each element. The y-axis of the scans was adjusted to permit a comparable scale. When visual comparisons were made of all the survey scans, no clear pattern emerged in regards to changes caused by AL irradiation.

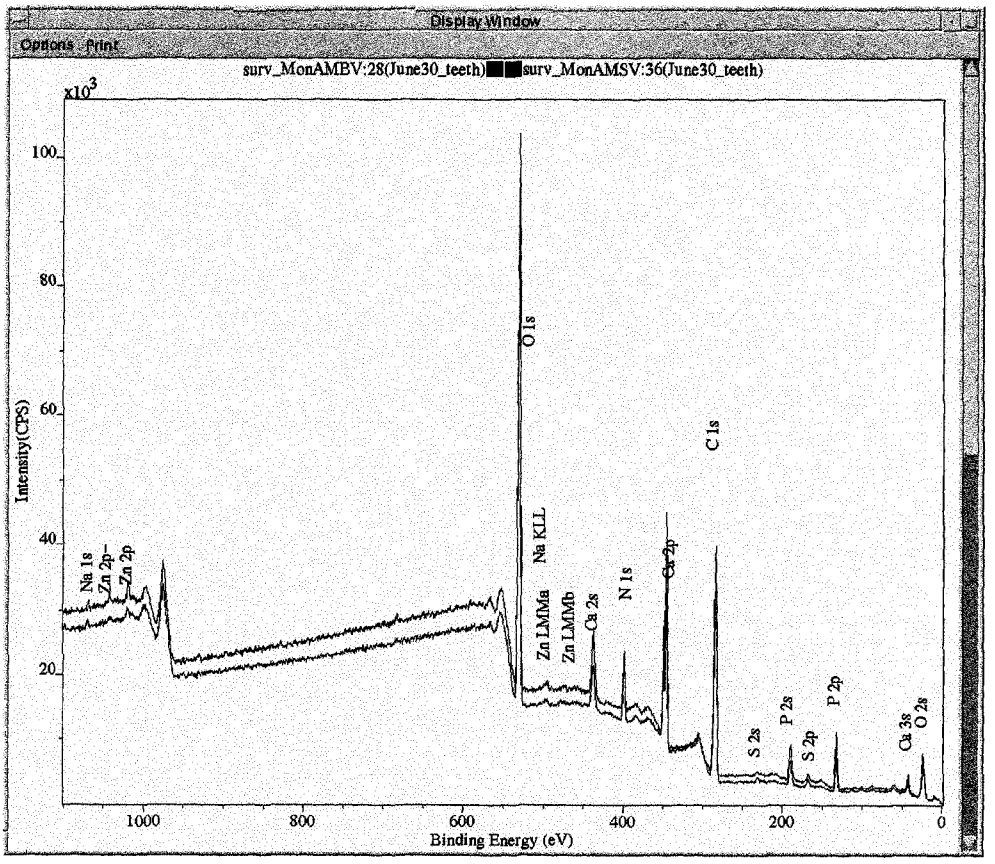

Fig. 2.10 Electron count plot versus Energy (energy spectrum) of Sample 1: Survey Scan (XPS). Peaks are displayed for Ca (2s and 2p), P (2s), O (1s), C (1s) and N (1s). This survey scan had characteristics typical of the spectra obtained in this study. Note that there is very little difference in the appearance of the scans, which suggests little chemical change occurred (control- blue; AL 10s- green). Table 2.2 below quantifies the main elements from the above survey scan.

Table 2.2: Quantification from Survey Scans for Sample 1

Atomic Conc %

Peak	control	10s	120s
O 1s	31.80	35.34	27.11
N 1s	6.36	5.29	5.36
Ca 2p	5.23	7.91	3.97
C 1s	53.13	46.25	59.02
P 2p	3.47	5.22	2.64

The C 1s peak was observed in all the samples examined. This peak was used for BE calibration by setting its BE to 285 eV to correct for sample charging. A further high resolution scan of the C 1s region revealed carbonate-type carbon was present in all samples. Carbon can be present in different chemical states due to the shifts in peak positions resulting from changes in the chemical structure and oxidation state of chemical compounds. The high resolution scan of carbon reveals that there are four different peaks present, which represent carbon at different binding energies (Fig. 2.11). The separation between these peaks allows differentiation of these four carbon species (CH; -CO; $-CO_2$; $-CO_3$). Such XPS peaks have been used previously to distinguish different species of the same element. Each peak area is proportional to the number of atoms being present in the studied element.[65]

Each of these carbon species is in a different chemical environment and therefore exhibits a different chemical shift. Carbon in the most electronegative environment (CO_3) appears at the highest binding energy, because the electronegative oxygen atoms withdraw electron density from the valence and bonding orbitals of the carbon atom. This reduces the screening of the core electrons from the nuclear charge and increases their binding energy.[64]

A sample scan for C 1s is displayed in Fig. 2.11. By examining the high resolution scan data and comparing the control to the experimental samples, it is seen there is no apparent visual difference in the carbonate concentration. The information garnered from the high resolution C 1s XPS scans was subsequently quantified (Tables 2.3,

2.4, 2.5, 2.6), to allow an assessment of carbonate concentrations between the different samples. To test for a statistically significant change in the carbonate concentration between the control and the samples irradiated with the AL (10 s or 120 s) a repeated measures ANOVA was performed. A repeated measures ANOVA gave a good statistical assessment, because this test allows the mean differences in percentage carbonate to be compared between the control, the 10s, and 120 s irradiated samples. There was no statistically significant change in the carbonate concentration with 10 s or 120 s of AL irradiation ($p>0.05$, Tables 2.7 and 2.8). Due to the small sample size non parametric tests were performed as well, and they also failed to detect any statistically significant change in the carbonate concentration, irregardless of the length of AL irradiation ($p>0.05$, Table 2.9).

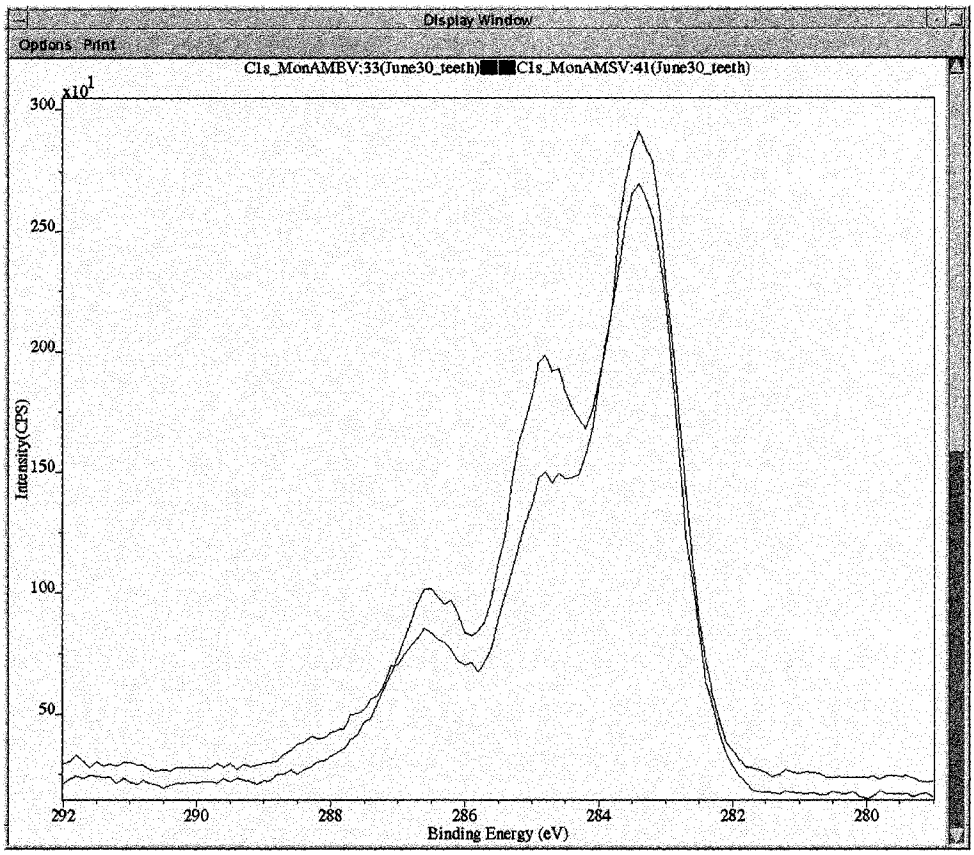

Fig. 2.11 Electron count plot versus Energy (energy spectrum) C 1s: High resolution Scan of Carbon (control blue, AL 10s green). The above spectra is representative of those acquired from all samples. The high resolution C 1s spectra were acquired at a 90 degree take-off angle for each sample. The above spectra are shown with the control and the sample irradiated for 10s overlayed. When overlaying XPS spectra a visual inspection is performed before quantification, to assess for any differences. If there is significant change in the number of peaks or movement along the x-axis, a chemical shift may have occurred. In the above overlay, there is very little change from the control to the experimental sample. There are several peaks, which represent different chemical states of carbon. The different states are from highest to lowest binding energy: CC-CH, C-O, O-C=O and CO_3. For examples of exact binding energies see tables 2.3, 2.4, and 2.5.

Table 2.3: Quantification from Components fitted to High Resolution Scans of Carbon for Sample 1 (Control)

Sample 1 (control)

Peak	Position BE (eV)	FWHM (eV)	Raw Area (CPS)	Atomic Conc%
C 1s CC,CH	283.4	1.21	5531.7	48.39
C 1s C-O	284.8	1.29	4008.5	35.06
C 1s O-C=O	286.5	1.16	1655.8	14.48
C 1s $-CO_3$	287.7	1.02	236	2.06

Table 2.4: Quantification from Components fitted to High Resolution Scans of Carbon for Sample 1 (Argon Laser Irradiation for 10s)

Sample 1 (10s)

Peak	Position BE (eV)	FWHM (eV)	Raw Area (CPS)	Atomic Conc%
C 1s CC,CH	283.4	1.18	5673.9	57.04
C 1s C-O	284.8	1.33	2788.1	28.03
C 1s O-C=O	286.5	1.13	1010.4	10.16
C 1s $-CO_3$	287.6	1.58	474.1	4.77

Table 2.5: Quantification from Components fitted to High Resolution Scans of Carbon for Sample 1 (Argon Laser Irradiation for 120s)

Sample 1 (120s)

Peak	Position BE (eV)	FWHM (eV)	Raw Area (CPS)	Atomic Conc%
C 1s CC,CH	282.9	1.16	11277.9	52.59
C 1s C-O	284.4	1.44	8702.2	40.58
C 1s O-C=O	286.2	0.84	677.1	3.16
C 1s $-CO_3$	286.9	1.23	786.2	3.67

Table 2.6: Descriptive Statistics for Percentage Carbonate (XPS)

Descriptive Statistics			
	N	SD	Mean
Percentage carbonate (control)	11	0.874	2.524
Percentage carbonate (10 s)	11	0.808	2.934
Percentage carbonate (120 s)	11	0.876	3.177

Table 2.7: Repeated Measures Pairwise Comparisons for Percentage Carbonate (XPS)

Pairwise Comparisons of Carbonate			
Treatment (I)	Treatment (J)	Mean Difference (I-J)	p-value
control	10 s	-0.410	0.266
	120 s	-0.653	0.108

Adjustment for multiple comparisons: LSD
P-value (sig.) of less than 0.050 is significant

Table 2.8: Repeated Measures ANOVA (XPS)

Multivariate Tests			
	Value	F	p-value
Wilks' Lambda	0.762	1.408	0.294

*Lambda ranges between 0 and 1, with values close to 0 indicating the group means are different and values close to 1 indicating the group means are not different

*P-value of less than 0.050 is significant

Table 2.9: Non-Parametric (Friedman) Test for Percentage Carbonate (XPS)

Test Statistics(a)	
N	**11**
Chi-Square	**4.55**
df	**2**
Asymp. Sig.	**0.103**

P-value of less than 0.050 is significant

In assessing the atomic concentration of other elements garnered from the survey spectra, there are a few points worth mentioning (Fig. 2.12). While assessing the carbonate concentration, data was automatically collected in regards to the concentrations of other elements. To better understand any other laser induced changes to the enamel, and open other avenues of research, these elements were also analyzed. These potential changes were not a primary focus of the study and due to the sample size the statistical significance of any results must be interpreted with caution. Table 2.10 displays the mean atomic concentrations of the 5 most common elements found in enamel. Repeated measures ANOVA was performed on all the elements comparing the concentrations of the control to the samples after 10 s and 120 s of AL irradiation. Most elements showed no statistically significant change, however, this was not always the case. The atomic concentration of nitrogen decreased from the control to the 120 s irradiated sample ($p < 0.05$, Table 2.10). This is a curiosity, but it is doubtful to play any significant structural role in the pretext of this study. Neither carbon nor oxygen showed a statistically significant change in their concentrations from the control to 10 s ($p > 0.05$). Even when further analysis was done at 120 s, no difference was found for carbon or oxygen. The percentage of

calcium slightly increased from the control to 10 s, but then decreased when the control was compared to the 120 s experimental sample ($p < 0.05$, Table 2.10). However, this likely does not reflect an actual change in the amount of calcium, but is an experimental artefact. *Chusuei et al.*[65] pointed out that this apparent decrease in the concentration of calcium with increased exposure to XPS is due to the instability of hydroxyapatite when it is exposed to the X-ray source. This leads to selective ejection of the calcium substituent, thus explaining the decrease with the second round of XPS testing. The phosphorous concentration increases from the control to 10 s ($p < .05$, Table 2.10), and then decreases slightly after 120 s of AL irradiation, although this latter change is not statistically significant.

Table 2.10: Quantification of Elements from Survey Scans

Mean Atomic Conc %						
Peak	N	control	10s	120s	p-value (10s)[a]	p-value (120s)[b]
O 1s	11	25.67	27.20	24.76	0.227	0.495
N 1s	11	8.36	8.57	6.56	0.688	0.013
Ca 2p	11	4.07	4.82	3.21	0.044	0.036
C 1s	11	59.24	56.20	61.66	0.060	0.245
P 2p	11	2.66	3.21	2.17	0.041	0.076

P-value of less than 0.050 is significant

a- p-values are a pairwise comparison of the control group to the 10 s AL irradiation group

b- p-values are a pairwise comparison of the control group to the 120 s AL irradiation group

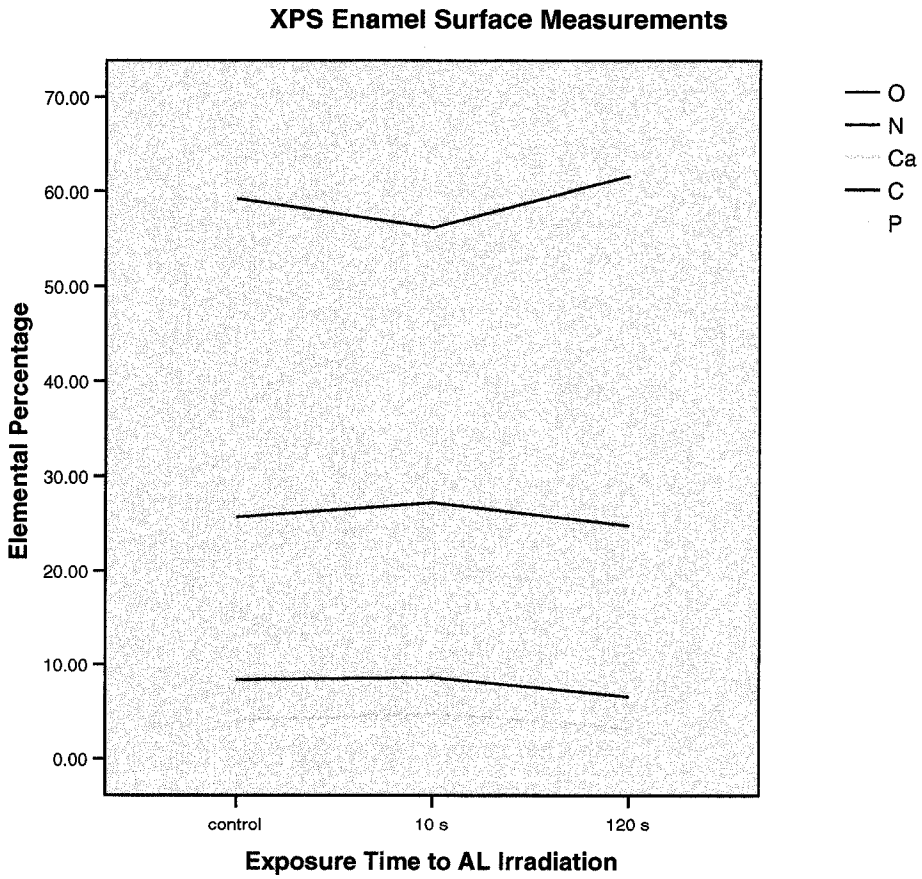

Fig. 2.12 Mean percentage composition (all 11 samples) of the tested elements in the enamel surface, as measured by XPS. There appears to be variations, however, not all are statistically significant (Table 2.10).

In regards to the changes seen in this study at 10 s of AL irradiation for calcium and phosphorous, it is doubtful they play any role in the surface characteristics of enamel. More important than small fluctuations in the measurements of calcium or phosphorous is the ratio between these two elements.[81] A decrease in this ratio signals a decrease in strength of the enamel surface. However, at no level of irradiation does a statistically significant change in the calcium/phosphorous ratio occur. This ratio of Ca/P has been used to distinguish different types of apatites. Pure hydroxyapatite has a Ca/P ratio of 1.67. Due to the impurities present in enamel, the Ca/P ratio has historically been found to be in the range of 1.48 ± 0.09.[80] This finding held true for the current study where the measured Ca/P ratios were consistently in this range (Table 2.11). There was a trend for the Ca/P ratio to fall with increasing exposure. This is not surprising since the calcium concentration was decreasing during XPS processing, as previously mentioned. However, the Ca/P ratio always stayed within the expected range for enamel hydroxyapatite. As well, a repeated measures ANOVA showed no statistically significant differences between the control groups and the AL irradiated samples at either 10 s or 120 s (Table 2.12).

Table 2.11: Calcium/Phosphorous Ratio (XPS)

Descriptive Statistics			
	N	Mean	SD
Ca/P control	11	1.541	0.086
Ca/P (10 s)	11	1.504	0.058
Ca/P (120 s)	11	1.478	0.079

Table 2.12: Repeated Measures Pairwise Comparisons for Calcium/Phosphorous Ratio (XPS)

Pairwise Comparisons			
Treatment (I)	Treatment (J)	Mean Difference (I-J)	p-value
control	10 s	0.037	0.330
	120 s	0.063	0.063

Adjustment for multiple comparisons: LSD
P-value of less than 0.050 is significant

2.3.2 Time Of Flight Secondary Ion Mass Spectrometry (ToF-SIMS)

Both positive ion and negative ion TOF-SIMS spectra of the enamel samples in the m/z 0-1000 range were acquired (Fig. 2.13). The characteristic peaks shown in the positive spectra include Ca^+ (m/z = 40), $CaOH^+$ (m/z = 57), CaO^+ (m/z= 56) and P (m/z= 31). The positive spectrum is dominated by the calcium ion at m/z 40 (Fig. 2.14). The characteristic peaks shown in the negative spectra include PO_3^- (m/z = 79), O^- (m/z = 16), PO_2^- (m/z = 63), and OH^- (m/z = 17), PO_4^-(m/z = 95), and for this study most importantly CO_3^-(m/z = 60). The important patterns in the negative ion spectra are the intensity variations of the CO_3^{-2} peak (Fig 2.15).

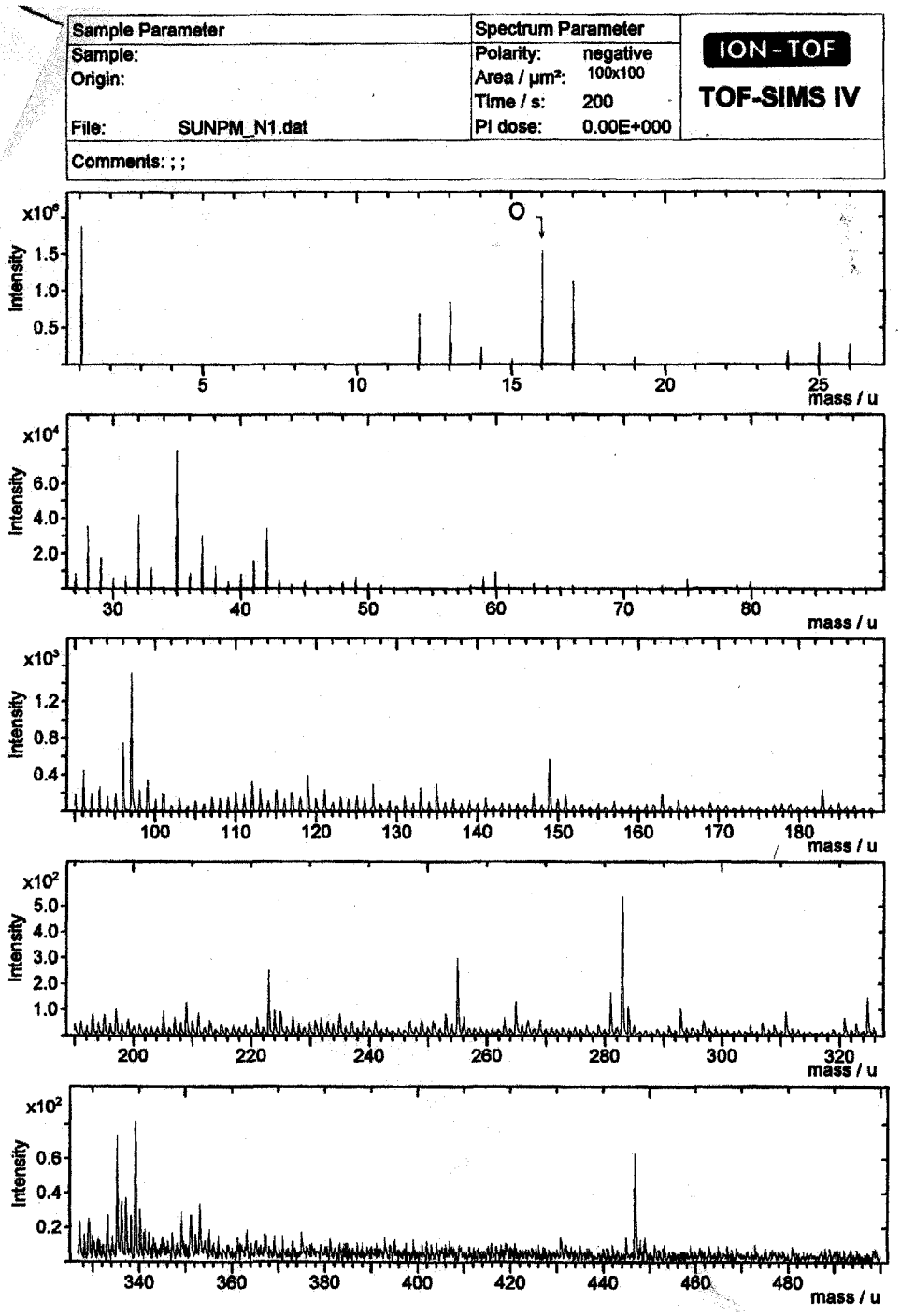

Fig. 2.13 Representative SIMS Survey Spectrum. The survey spectra are from m/z 0-1000 although only up to 500 is displayed here. The ions of interest are in the lower m/z range, so these spectra were examined, but not used extensively. One of the following spectra was taken for each sample in positive and negative polarity.

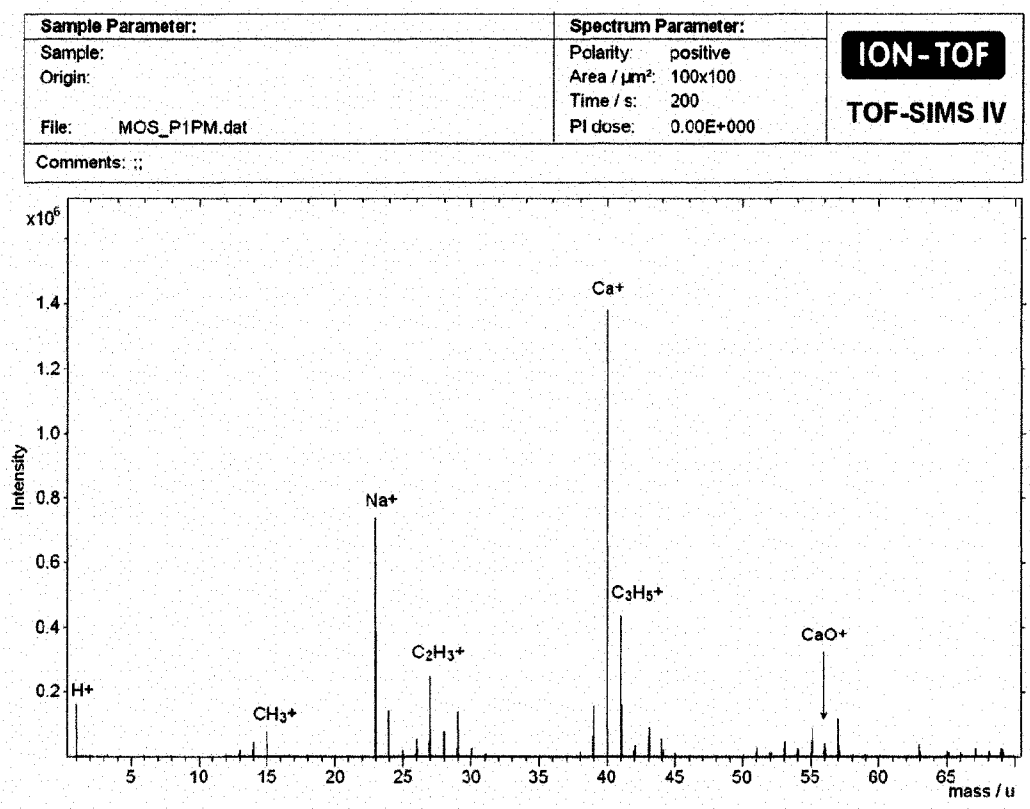

Fig. 2.14 Representative SIMS High Resolution Positive Spectra. An initial spectra was taken on all samples from m/z 0-1000, but the region from m/z 0-100 was then concentrated on. The ToF-SIMS spectra display the relative yield (the number of secondary ions detected per incident primary ion) versus secondary ion mass. Each spectrum was acquired from the enamel surface with a primary ion dose, for each projectile, of not more than 10^{12} ions cm^{-2}. The secondary ions are separated by mass and charge. The above spectra is for positive charged ionic species, which can be monoatomic or polyatomic. Similar characteristic peaks appeared in most of the samples, but with some changes in relative peak intensities. The diagnostic positive ions of hydroxyapatite in the spectra are observed at m/z 23, 40, 56 and 57 corresponding to Na^+ Ca^{+2}, CaO^+ and CaOH. Note that the calcium ion at mass number 40 has the greatest intensity, this was a common finding in the samples.

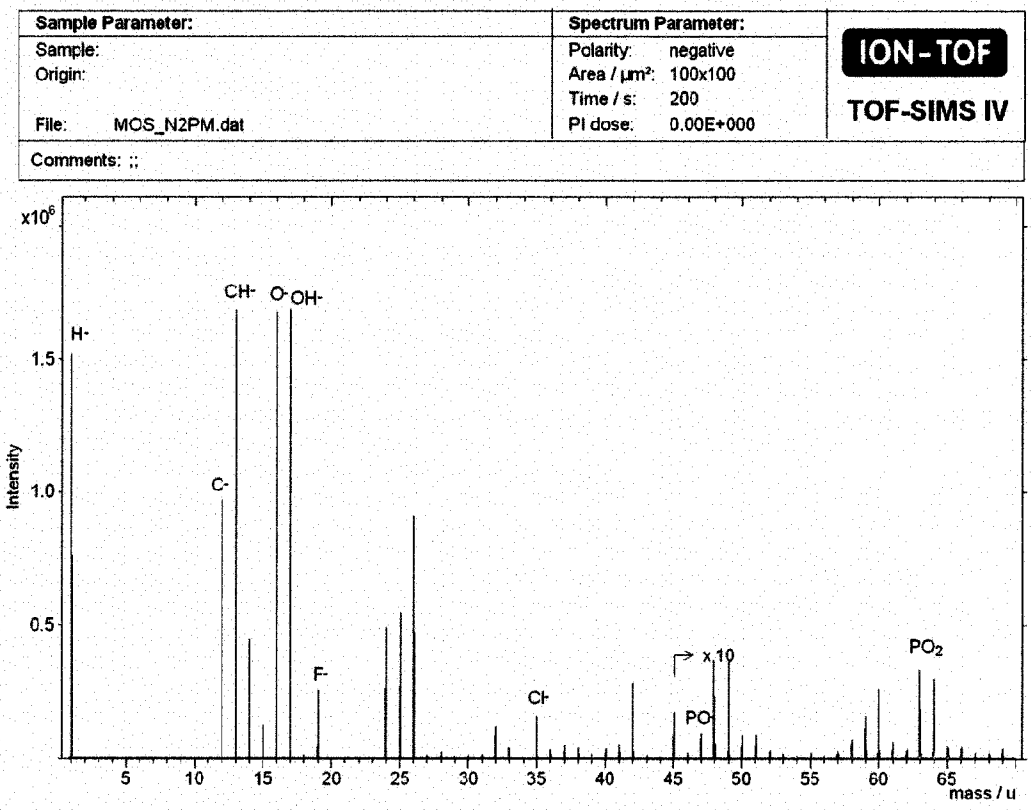

Fig. 2.15 Representative SIMS High Resolution Negative Spectra. An initial spectra was taken on all samples from m/z 0-1000, but the region from m/z 0-100 was then concentrated on. The ToF-SIMS spectra display the relative yield (the number of secondary ions detected per incident primary ion) versus secondary ion mass. Each spectrum was acquired from the enamel surface with a primary ion dose, for each projectile, of not more than 10^{12} ions cm^{-2}. The secondary ions are separated by mass and charge. The above spectra is for negative charged ionic species, which can be monoatomic or polyatomic. Although not labeled above, this study was interested in the polyatomic ion carbonate (CO_3^{-2}). Similar characteristic peaks appeared in most of the samples, but with some changes in relative peak intensities. The diagnostic negative ions of hydroxyapatite in the spectra are observed at, O^- (m/z = 16), OH^- (m/z = 17), PO_2^- (m/z = 63) PO_3^- (m/z = 79), PO_4^-(m/z = 95), and CO_3^-(m/z = 60). The above spectra ended at 70, however, it was available for data collection well past the m/z number of 95 for phosphate.

Since static SIMS is a qualitative rather than quantitative technique, the most important choice in deciding upon which fragment ion to select was the uniqueness of that fragment for identification purposes.[82] Although SIMS is not considered to be a truly quantitative technique, a certain level of quantification is possible through ion intensity ratios. A peak area ratio analysis of calcium and carbonate was performed on the ions at m/z 40 and m/z 60 to ascertain any changes occurring in the relationship between the two ions (Table 2.13). The exact mass number for carbonate using SIMS is 60.0092 and for calcium 40.078. Additionally the ratio of carbonate to phosphate (m/z 94.971) was performed and compared from the control to the experimental sections. When mass numbers are recorded from SIMS there are often small variations in the recordings for a given ion, due to surface charging or stray atomic species disrupting the time of flight. Thus, for the present analysis the ionic count for the above mass numbers for calcium and carbonate ± 0.1% were included in the calculations.

Table 2.13: Ratio of Carbonate to Ca^{40} ionic counts (SIMS)

Descriptive Statistics			
	N	**Mean**	**SD**
Carbonate/Ca (control)	**11**	**0.134**	**0.389**
Carbonate/Ca (10s)	**11**	**0.007**	**0.006**
Carbonate/Ca (120s)	**11**	**0.095**	**0.168**

Due to the fact each tooth had been sectioned in two, this consisted of a paired sample to be studied. One section was the control and the other was irradiated with an AL for 10 s. As an adjunct in this study, the experimental portion of the tooth was further irradiated for 110 seconds (for a total of 120 s). This exaggerated any changes that the AL could produce. A repeated measures ANOVA allowed a comparison of the mean carbonate/calcium ratios from the control group, to 10 s, to 120 s. The repeated measures test showed no change in the carbonate concentration irregardless of the time of AL irradiation ($p>0.05$, Tables 2.14 and 2.15). Non parametric tests were also performed on the above data set, and this again elucidated no statistically significant differences ($p > 0.05$, Table 2.16). As an aside, since it is known that the carbonate ion substitutes for the phosphate ion, a comparison of the ionic counts of these two ions was performed with the SIMS data. Once again, there was no statistically significant difference between the control and experimental samples, when using repeated measures ANOVA (Tables 2.17 and 2.18; Appendix F).

Table 2.14: Repeated Measures Pairwise Comparisons of Ratio of Carbonate to Ca^{40} ionic counts (SIMS)

Pairwise Comparisons of Carbonate/Phosphate Ratio			
Treatment (I)	**Treatment (J)**	**Mean Difference (I-J)**	**p-value**
control	**10 s**	**0.127**	**0.307**
	120 s	**0.039**	**0.658**

P-value of less than 0.050 is significant
Adjustment for multiple comparisons: LSD

Table 2.15: Repeated Measures ANOVA (SIMS)

Multivariate Tests				
Effect		**Value**	**F**	**p-value**
Carbonate/Ca Ratio	**Wilks' Lambda**	**0.752**	**1.485**	**0.277**

*Lambda ranges between 0 and 1, with values close to 0 indicating the group mean are different and values close to 1 indicating the group means are not different

*P-value of less than 0.050 is significant

Table 2.16: Non-Parametric (Friedman) Test for Carbonate/Ca ratio (SIMS)

N	**11**
Chi-Square	**2.182**
df	**2**
Asymp. Sig.	**0.336**

P-value of less than 0.050 is significant

2.4 Conclusions

1. No statistically significant difference was found in the carbonate concentration of enamel after exposure to AL irradiation (10 s) as tested by XPS. Therefore the null hypothesis is not rejected.
2. No statistically significant difference was found between the carbonate ionic counts of enamel after exposure to AL irradiation (10 s) as tested by ToF-SIMS. Therefore the null hypothesis is not rejected.

As well, there were no significant differences in the concentration of carbonate after 120 s of AL irradiation, as measured by XPS or ToF-SIMS. Additionally there was minimal change in the chemical composition of enamel as a whole with either 10 s or 120 s of AL irradiation as measured by XPS or ToF-SIMS.

In summary, the ToF-SIMS and XPS surface scans displayed that the calcium hydroxyapatite in human enamel has a significant carbonate component. However, the amount of carbonate does not appear to be reduced by AL irradiation. A limitation of this study was the small sample size. Due to the lack of research *a priori* and taking into account the time and cost restrictions of the equipment involved an arbitrary sample size of 11 was arrived at. Further research into assessing carbonate changes in the enamel after exposure to AL irradiation can have a more accurate sample size due to the information gathered in this study. An acceptable clinical goal would be to change the carbonate concentration by 1 % or higher. Analyzing the change in the mean carbonate concentrations from the control to the samples

irradiated with the AL for 10 s with a paired t-test gives a standard deviation of 1.15. Using this standard deviation with a significance level of 0.05 and a power of 0.9 the sample size for future research should be at least 16, to detect a 1 % change or greater in the carbonate concentration.[83]

Chapter 3: Discussion and Recommendations

Use of the argon laser in orthodontic practice has yet to gain widespread clinical acceptance. Although the AL definitely has its advantages over the CCL, such as reduced curing times and no loss of power at greater distances from the tooth, it is far more expensive and takes up considerably more space than CCL. As it stands now, it would seem more benefits of the AL need to be uncovered for it to gain a wider acceptance by practicing clinicians.

In the past one such advantage was the large time saving that the AL provided as opposed to the CCL. However, in the last few years other high speed curing lights have been introduced. The blue light emitting diode (LED) curing light based on gallium nitride technology is an example of such a light. The spectral output of the LED falls mainly within the absorption spectrum of the camphorquinone in dental composites (95% between 400 and 500 nm) with a peak wavelength of 470 nm.[84]

LED's have several positive features, which are leading to their increased usage in orthodontic settings. The lights are small, cordless and therefore easy to move around the office. The LED is very efficient and generates little heat, thus no fan is required during operation. Due to the precise nature of the emitted light, only 10 s of curing is needed per tooth with the LED. In addition the LED requires less than 10 percent of the electrical power consumed by CCL. *Mills et al.*[84] presented results that demonstrate the capability of LED to cure in a significantly greater depth for three different types of composite than a CCL. *Jandt et al.*[85] found that the compressive strengths of dental composites cured under laboratory conditions with a LED were not statistically different to those cured with a CCL. The recent development of the

LED with its many advantages has allowed it to become the curing light of choice in many orthodontic offices. The previous advantage of the AL as opposed to the CCL was the potential for time savings. However, the LED has negated this advantage, and it is far more convenient to use than the AL. For the AL to be widely adopted it must be able to offer positive traits not found in the LED.

One such advantage could be The AL's potential to increase the demineralization resistance of enamel. This increased resistance is an attribute the LED does not claim to possess. One of the biggest problems facing clinicians is the appearance of "white spot" demineralized lesions on the enamel surface surrounding orthodontic brackets. A device which would reduce the appearance of these lesions would be looked upon very favorably by orthodontists and patients alike. Recent studies have supported the claim that the AL increases the demineralization resistance of enamel.[4-8] However, none of these studies duplicated the conditions found intraorally with orthodontic patients. These studies showed a reduction in the demineralization of enamel, but if the AL just physically modified the surface, these possible surface changes could be reversed over the following several years of orthodontic treatment. Therefore any increase in demineralization resistance may only be temporary, and not provide protection over the course of orthodontic care. However, if it could be shown that the AL permanently altered the chemical structure of enamel in a way that increased its resistance to demineralization, this would be an important finding. It would also add credence to the argument that any increased demineralization resistance was in fact permanent.

A reduction in the carbonate content of enamel makes it more resistant to demineralization. The mechanical and chemical properties of enamel are very sensitive to chemical substitutions, such as the replacement of phosphate with carbonate in the hydroxyapatite crystals, which form enamel. This substitution of carbonate affects enamel properties by changing the hydroxyapatite crystal structure resulting in an increased lattice strain, decreased crystal size and increased solubility. Properties on a crystallite level, such as growth, solubility and diffusion, are considered to be basic in many aspects of dental development and health, because as a result of these crystallite changes, the enamel surface is less resistant to the process of demineralization, and more prone to developing caries.

Time-of-flight secondary ion mass spectrometry (ToF-SIMS) and X-ray photoelectron spectroscopy (XPS) analyses were used to investigate the carbonate content of enamel before and after AL irradiation. The surface analytical data suggests that there was no change in the carbonate concentration of enamel after 10 s of AL irradiation at 250 mW. Comparison of relative ion intensities in the ToF-SIMS spectra revealed no statistically significant change in the carbonate concentration of enamel. As well, increasing the AL irradiation to 120 seconds still produced no apparent change in the carbonate content of enamel. ToF-SIMS and XPS were both in agreement in this finding.

The XPS and ToF-SIMS analysis of the enamel provided a detailed insight into the

carbonate content of the enamel before and after AL irradiation. The data presented demonstrates the potential of these surface science techniques to examine elemental changes in tooth structure. This study is of significant value from a biomedical perspective and serves as an addition to the XPS and ToF-SIMS literature. Also, it should serve as a basis for future studies, to permit elemental assessment of potential changes in tooth structure after being subjected to the wide range of processes used in orthodontics. Only by elemental examination, can the effect of a given procedure on tooth structure truly be ascertained. This knowledge will lead to a greater understanding of elemental changes, and consequently possible technical improvements to clinical techniques may follow.

Since the AL seems to have little effect on the carbonate concentration at clinical time and power settings, it must be ascertained if the AL imparts caries resistance and if this is in fact permanent. A long term split mouth designed study would be required to study this question.

A broad consensus exists that the presence of carbonate makes enamel more susceptible to demineralization. Avenues of research should also be explored to assess means of reducing carbonate levels. These methods must be practical in a clinical setting or they will never gain acceptance. One such way may be to develop a laser, which can operate both as an argon laser and a carbon dioxide laser. *Featherstone et al.*[45] found that during irradiation with a carbon dioxide laser, the heat generated causes carbonate loss from the carbonated hydroxyapatite enamel,

converting it to a low solubility hydroxyapatite-like calcium phosphate, and this occurred in less than 4 seconds. The carbon dioxide laser operates in the infrared spectrum at a wavelength of 10.6 μm, while the argon laser is in the blue-green spectrum (.457μm-.514μm). The laser wavelength is the primary determinant of the extent to which light is absorbed by a particular tissue. For enamel, the wavelengths which are strongly absorbed by the enamel are in the range of 7-11μm.[29] So in the case of caries prevention the laser interaction will most likely need to change the mineral from its acid soluble form to a much less soluble form. Light that is not specifically absorbed in enamel will be extremely inefficient and energy densities at high levels, or for increased lengths of time will be required to have any effect at all. The band at around 7μm is where the carbonate ion that substitutes in dental mineral for phosphate absorbs light. In the region of 9-11μm the primary absorber is the phosphate ion. Therefore a laser with a wavelength of 9-11μm wavelengths is likely to have a rapid and major effect on the mineral, giving the most efficient conversion of light to heat in the mineral of the enamel.

It would be interesting to know if a reduction of carbonate occurred with a carbon dioxide laser at settings which were practical and safe clinically. If this were the case, a quick cure of the composite resin with the argon laser, could be followed by less than 5 seconds of curing with the carbon dioxide laser. If this could be accomplished with one device and for limited amounts of time, this may provide significant reduction in enamel demineralization. Orthodontists would be very receptive to a practical solution to this problem, especially if it could be accomplished

in a manner which did not rely on patient compliance.

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Appendix A

Health Research Ethics Board

213 Heritage Medical Research Centre
University of Alberta, Edmonton, Alberta T6G 2S2
p.780.492.9724 (Biomedical Panel)
p.780.492.0302 (Health Panel)
p.780.492.0459
p.780.492.0839
f.780.492.7808

HEALTH RESEARCH ETHICS APPROVAL

Date:	March 2006
Name of Applicant:	Dr. Paul Major
Organization:	University of Alberta
Department:	Graduate Orthodontics
Project Title:	**Argon Laser Induced Changes to the Surface Properties of Enamel**

The Health Research Ethics Board (HREB) has reviewed the protocol for this project and found it to be acceptable within the limitations of human experimentation. The HREB has also reviewed and approved the subject information letter and consent form, if applicable.

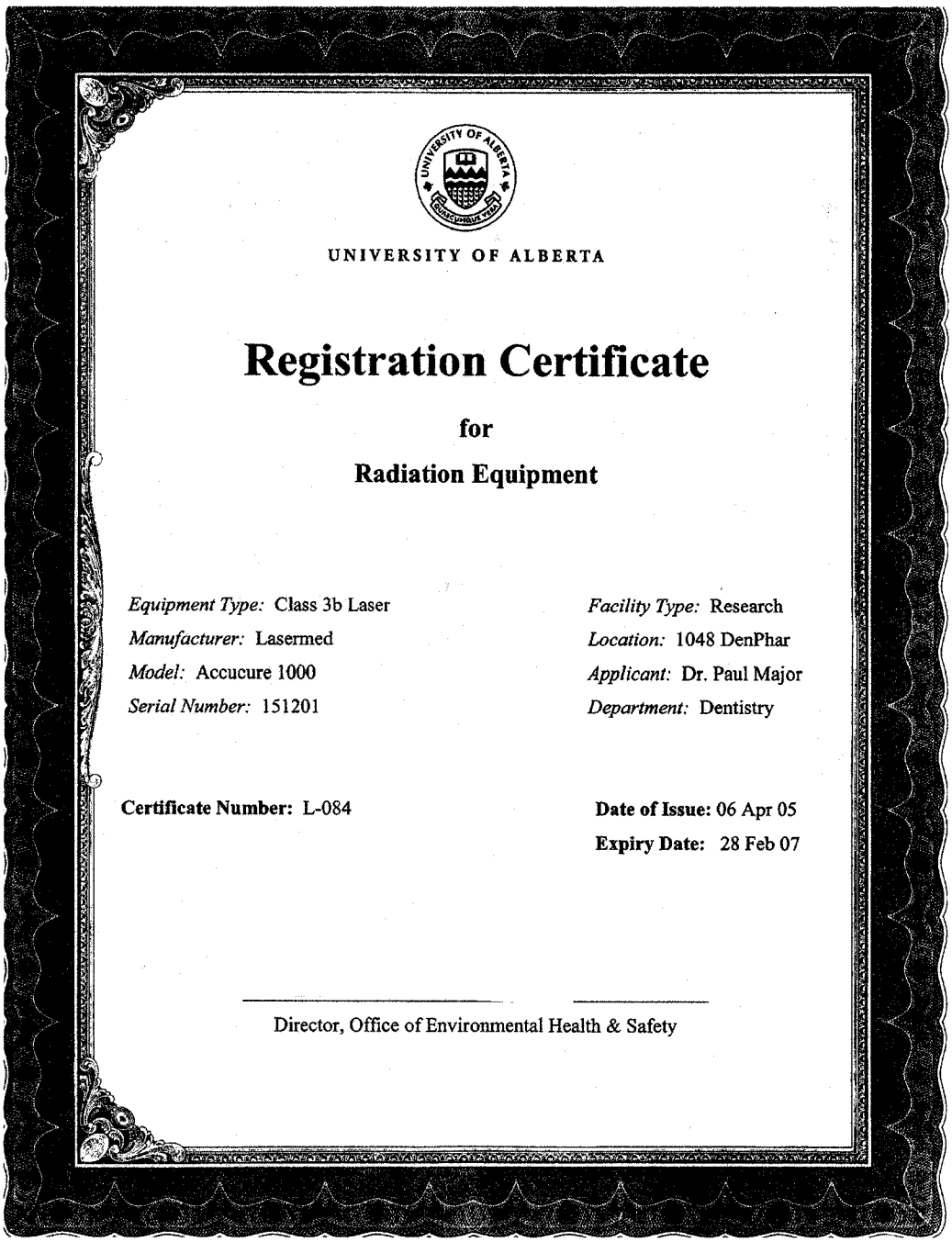
The approval for the study as presented is valid for one year. It may be extended following completion of the yearly report form, which will be sent to you in your renewal month. Any proposed changes to the study must be submitted to the Health Research Ethics Board for approval. Written notification must be sent to the HREB when the project is complete or terminated.

Dr. Glenn Griener
Chair of the Health Research Ethics Board
(B: Health Research)

File number: B-220305

UNIVERSITY OF ALBERTA Capital Health CARITAS HEALTH GROUP

Appendix B



UNIVERSITY OF ALBERTA

Registration Certificate

for

Radiation Equipment

Equipment Type: Class 3b Laser
Manufacturer: Lasermed
Model: Accucure 1000
Serial Number: 151201

Facility Type: Research
Location: 1048 DenPhar
Applicant: Dr. Paul Major
Department: Dentistry

Certificate Number: L-084

Date of Issue: 06 Apr 05
Expiry Date: 28 Feb 07

Director, Office of Environmental Health & Safety

Appendix C

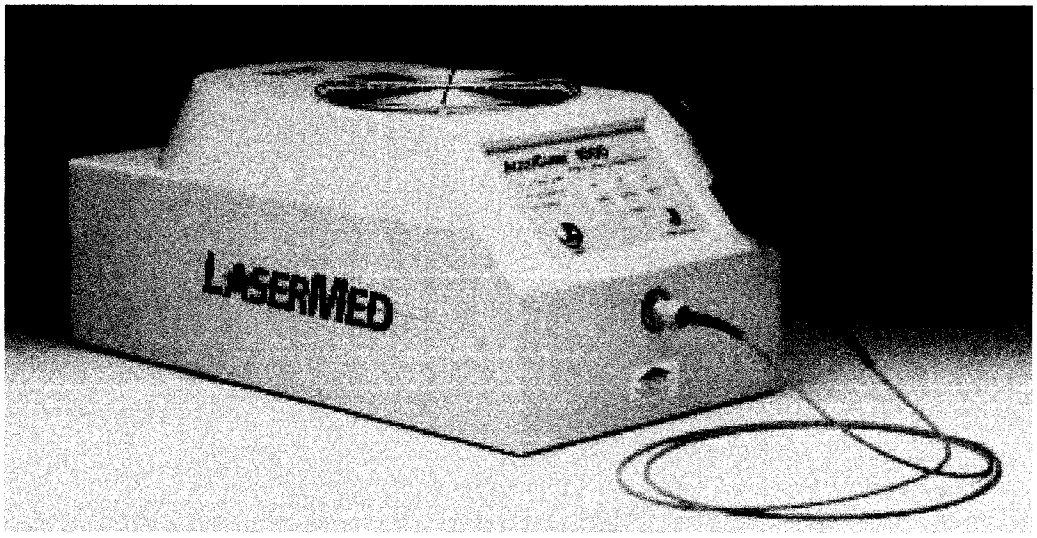


Figure 2.1 Argon Laser (AccuCure 1000, Salt Lake City, UT)

Technical Specifications:

Laser Type:	Argon Ion Air Cooled
Laser Tube Type:	Internal Mirror, Ceramic/Metal
Wavelength:	457nm – 502 nm
Delivery System:	Fused Silica Fiber Optic UltrLite ABS Plastic
Handpiece	
Power Control Selectable:	150 mW – 250mW in 50mW increments
Laser Tube Average Life:	2000 Hours
Voltage:	120 VAC
Current:	20 amp
Unit Dimensions:	20.4 cm X 40.7 cm X 17.8 cm
Unit Weight:	8.2 kg

Appendix D
Authorization for Laser Usage

UNIVERSITY OF ALBERTA
Office of Environmental Health & Safety
Radiation Safety
11390 – 87 Avenue, Rm 107 Ed Car Park
Edmonton, Alberta, Canada T6G 2R5
www.ehs.ualberta.ca
carl.schumaker@ehs.ualberta.ca
Tel: 780.492.5655
Fax: 780.492.7790

March 2, 2005

To: Michael Ziglo
Graduate Orthodontics
1st Floor Dentistry Pharmacy

From: C.D. Schumaker
Radiation Protection Officer
Office of Environmental Health & Safety

Reference: Laser Safety Training & Baseline Eye Examination

This letter acknowledges your completion of the requirements for laser safety training (01 Feb 05) and a baseline eye examination (26 Feb 05) prior to work with Class 3b or 4 lasers at the University of Alberta. You are now an authorized laser user.

Carl D. Schumaker, MSc

Appendix E
Letter of Consent

April 22, 2005

Title of Study: **Argon Laser Induced Changes to the Surface Properties of Enamel**

Investigators:
- Dr. Michael Ziglo (Orthodontics) (780) 492-4469
- Dr. Paul Major (Orthodontics) (780) 492-7696
- Dr. Alan Nelson (Engineering) (780) 492-7380
- Dr. Gison Heo (Orthodontics) (780) 492 4469

You are being asked to participate in a study at the University of Alberta.

Purpose of the Study:

Orthodontics requires placing special brackets on the teeth, to then move the teeth. However, there can be problems with attaching brackets to teeth. One of the most common problems is the small white spots that can appear around the edges of the brackets. These spots are both unsightly and can lead to cavities. If there was a way to strengthen the enamel, this would hopefully reduce these spots. An Argon Laser can be used to attach the brackets to the teeth. It is also thought to change the enamel and make it stronger. This would hopefully reduce the number of spots appearing on the teeth. The main goal of this study is to gain information regarding the changes to the enamel as a result of using the Laser.

Your permission is being requested to use sample of enamel from your extracted teeth (3^{rd} molars). These teeth normally would be thrown away. Permission to use your extracted teeth does not pose any risk to you. This study is completely voluntary, and choosing not to have your teeth included in the study will not affect your treatment in any way.

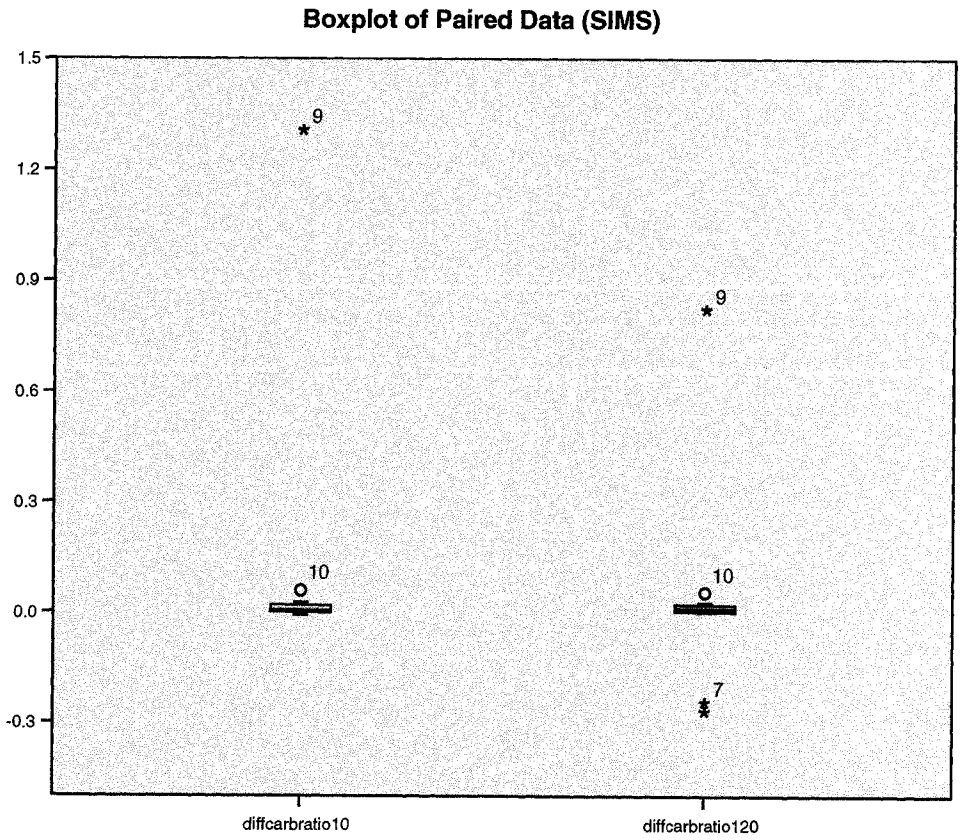
No personal information about you will be included with the teeth. Also, only the enamel surface will be used for this study, the rest of the tooth will be discarded. Since the enamel contains no DNA, no DNA information will be known to the researchers. The teeth will be destroyed on completion of this study. Should you have any concern about any aspect of this study, you may contact Dr. Michael Ziglo at (780) 492 4469.

By signing below, I agree to allow a sample of the enamel from my 3rd molars to be used for research purposes as outlined in the information letter.

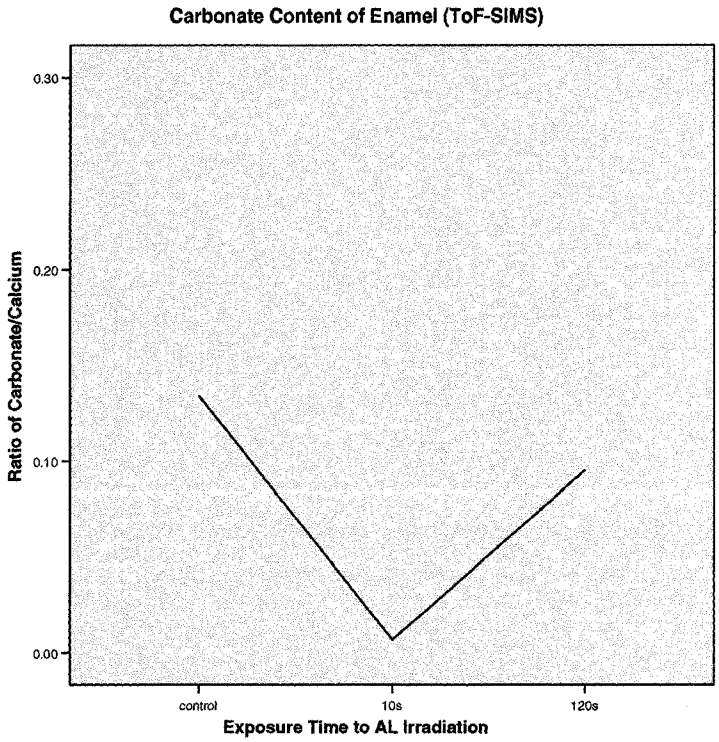
Patient Signature: _____

Patient Name: _____ Date: _____

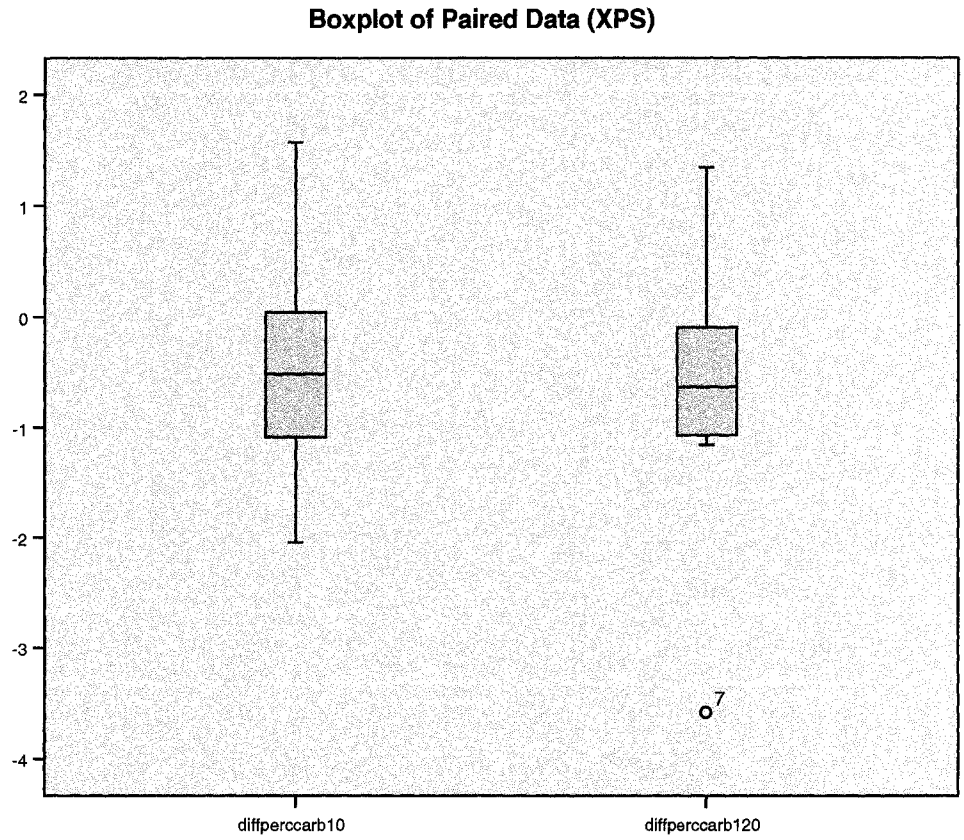
Appendix F



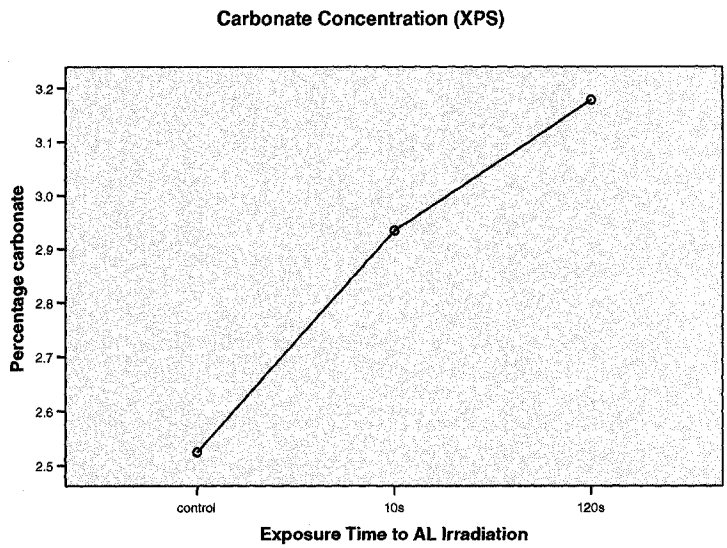
The above box plots give us a univariate comparison of each group of paired data. The Y axis is the ratio of carbonate/calcium from the SIMS data. The X axis is the ratio difference between either the control and the 10s irradiated sample, or the control and the sample irradiated for 120s. In the 1st box plot, diffcarbratio10, it is somewhat difficult to extract much information. There are two potential outliers (points 9 and 10). This boxplot represents the difference between the ratio of carbonate/calcium in the control minus the experimental sample cured with the AL for 10s. The second boxplot compares the ratio between the control and the experimental section cured with the AL for 120s. The second boxplot is also difficult to assess, and again has potential outliers (points 7, 9, and 10). Both of these cases were analyzed with and without the outliers, and this produced no statistical difference. Therefore the outliers were left in. From these boxplots normality cannot be ascertained. However, a smaller sample size was a limitation of this study from the outset. To compensate, parametric and non-parametric statistics were run on this data.



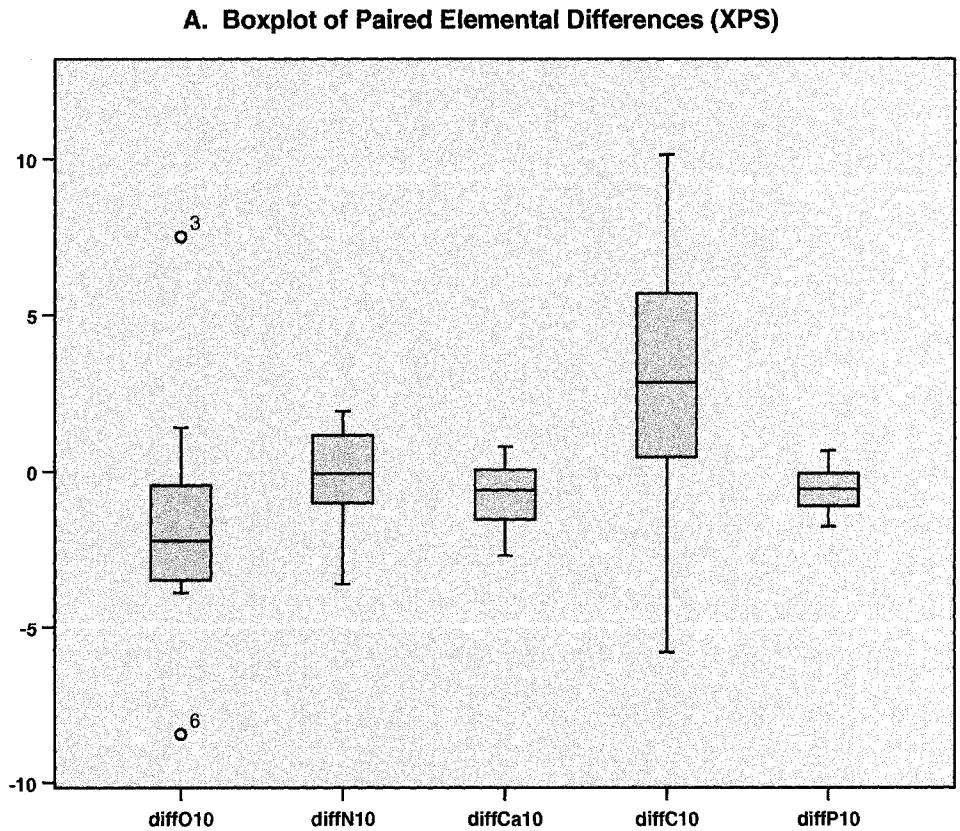
The above graph shows that in there appears to be no consistent change in the carbonate content of enamel when exposed to AL irradiation.



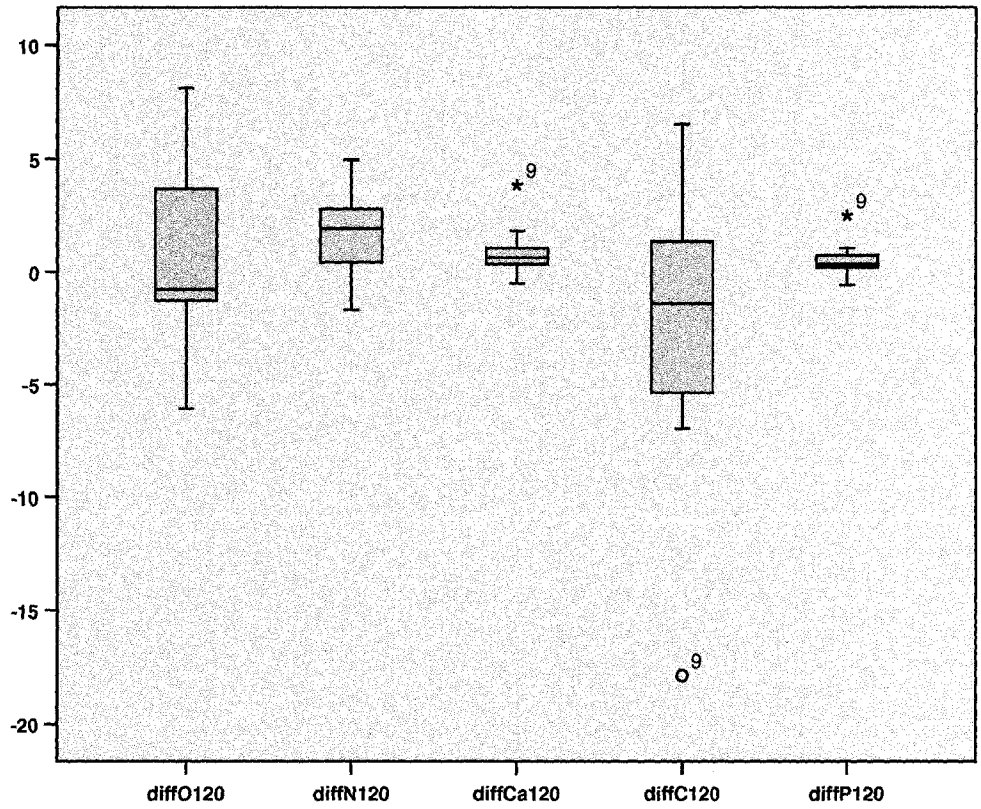
The above boxplot allows us a univariate look at the XPS data. The Y axis units are percentage composition of each element in enamel. As we can see, individually there is some variation in the standard deviations, and skewness. However, we will assume normality within each paired data group.



The above graph show that there is a trend for an increase in carbonate concentration, however, this is not statistically significant.



B. Boxplot of Paired Elemental Differences (XPS)



The above two boxplots allow a look at the XPS data for the major elements present in the enamel surface. The units on the Y axis are a percentage, and represent the percentage difference in the control enamel surface compared to the 10s sample (A) or the 120s sample (B). The X axis abbreviations are (in order from left to right) for the difference in oxygen, nitrogen, calcium, carbon, and phosphorous.

Individually in the boxplots there is some variation in the standard deviations and skewness. As well, there are several potential outliers present. All data was statiscally analyzed with and without the potential outliers, and there was found to be no difference. With this particular data normality will be assumed in the statistical analysis.

Table 2.17: Repeated Measures Pairwise Comparisons of the Ratio of Carbonate to Phosphate ionic counts (SIMS)

Pairwise Comparisons of Carbonate/Phosphate Ratio			
Treatment (I)	**Treatment (J)**	**Mean Difference (I-J)**	**p-value**
control	**10 s**	**-984.166**	**0.254**
	120 s	**-1537.385**	**0.192**

P-value (sig.) of less than 0.050 is significant
Based on estimated marginal means
Adjustment for multiple comparisons (LSD)

Table 2.18: Repeated Measures ANOVA of Ratio of Carbonate to Phospate (SIMS)

Multivariate Tests			
	Value	**F**	**p-value**
Wilks' lambda	**0.640**	**1.965**	**0.210**

*Lambda ranges between 0 and 1, with values close to 0 indicating the group means are different and values close to 1 indicating the group means are not different
*P-value of less than 0.050 is significant

Appendix G
Raw data

XPS

Tooth	% Carbonate (control)	% Carbonate (10s)	% Carbonate (120s)
1	1.09	2.21	2.17
2	2.15	3.21	1.74
3	2.93	3.01	3.19
4	2.37	2.83	3.2
5	3.22	1.71	3.64
6	2.18	2.7	2.81
7	1.04	3.08	4.62
8	2.66	3.53	3.73
9	3.13	2.96	3.04
10	3.81	2.24	2.47
11	3.17	4.8	4.33

SIMS

	Ionic Counts					
Tooth	Carbonate (control)	Carbonate (10s)	Carbonate (120s)	Ca40 (control)	Ca40 (10s)	Ca40 (120s)
1	16949	11250	46	1654906	1896351	1221188
2	32347	17	7	1252935	232245	352800
3	19054	1269	7	1328567	440937	12348
4	9833	2739	48	996556	1000773	7881
5	14970	14732	1	1599015	1448448	236679
6	3380	46	268	1080221	473273	82297
7	693	15553	9403	41264	912418	35875
8	4648	1588	245	1077952	133318	520507
9	1341	598	2759	1026	298399	5666
10	116649	30863	37	1663869	2473309	2287
11	4670	1532	19335	1056592	116651	70515