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Structural Changes on Human Dental Enamel Treated with Er:YAG, CO₂ Lasers and Remineralizing Solution: EDS Analysis

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1. Introduction

It has been reported that common lasers employed for caries prevention on the enamel surface include CO₂ and Er:YAG types (Ana, et al., 2006); however, CO₂ lasers appear to be more appropriate for preventing dental caries (Rodrigues, et al., 2004). The irradiation of teeth with a laser results in an interaction between the light and the biological constituents of the dental hard substance. In the case of absorption by the specific components of the dental enamel, the irradiated energy is converted directly into heat. This thermal effect is the cause of the structural and chemical changes occurring in the enamel after laser irradiation (Apel, et al., 2002).

The association of laser irradiation with fluoride has demonstrated the best results in the inhibition of caries development. The combined treatment of laser irradiation with fluoride propitiates an expressive fluoride uptake, reducing the progression of caries-like lesions, and this treatment is more effective than laser or fluoride alone. The available data suggest that lasers combined with fluoride are a promising treatment in caries prevention (Ana, et al., 2006). In the same way, the effect of remineralizing solution has been evaluated on caries formation and its progression in the primary tooth enamel. Remineralizing solution has been considered an effective measure for the prevention of white spot lesions because it has the ability to provide mineral sources of calcium (Ca), phosphate and fluoride to demineralized or hypomineralized enamel surfaces, making them less soluble during cariogenic challenges (Westerman et al., 2006). Caries prevention has been evaluated by several techniques, including atomic absorption spectrometry (to obtain Ca and P (phosphorus) concentrations liberated into acid solution), polarized light microscopy (to determine lesion depth) and SEM (to examine morphological changes). However, only minimal research has focused on enamel chemical composition after laser irradiation and acid dissolution. For this purpose, FT-Raman microscopy, X-ray photoelectron spectroscopy (XPS) and energy dispersive spectroscopy (EDS) have been used.

Energy dispersive spectroscopy (EDS) is an analytical technique that allows the detection of the elements present in the studied material. It is very versatile and can be used with any type of solid sample, from metals and ceramics to biological tissues. It is usually coupled with an electron microscope to simultaneously observe the exact area of interest from which the signal will be collected.

EDS analysis provides a specific method to determine the concentration of chemical elements on substratum surfaces, and it has been largely used in engineering and chemistry, but its application has not been widespread in dentistry (Paradella et al., 2008). At first, EDS analysis was introduced into dental research for the basic characterization of restorative dental materials used to repair damaged teeth and/or replace missing teeth (Marshall et al., 1988). More recently, this technique has been used to assess structural changes in tooth surfaces produced by the application of dental materials (Caltabiano, et al., 1996; Papagiannoulis, et al., 2002; Rosin-Grgewt, et al., 2006; Weerasinghe, et al., 2007; Della, et al., 2008; Markovic, et al., 2008; Paradella, et al., 2008; Scougall-Vilchis, et al., 2009; Keinan, et al., 2010) and of laser irradiation (Rodríguez-Vilchis, et al., 2010; Rodríguez-Vilchis, et al., 2011).

The purpose of this chapter is to present the use of EDS as a useful technique for dental research, taking as references previous reports on this area of the knowledge. Additionally, the authors present original research, based on the application of EDS to assess the microstructural changes on the enamel surface achieved through the application of two types of dental lasers and a remineralizing solution, to avoid the development of white spot lesions as a side effect of orthodontic treatment.

2. Enamel structure

To comprehend the nature of the structural changes in human dental enamel treated with Er:YAG and CO₂ lasers and remineralizing solution, it is important to understand the structure of untreated human dental enamel.

Tooth enamel, the hardest mineralized human tissue, (Harris & García-Godoy, 1999; Wang, et al., 2005), is composed almost exclusively (more than 95 wt%) of hydroxyapatite (Ca₁₀(PO₄)₆(OH)₂, HAP) (Pan, et al., 2008), with incorporated trace elements (Reitznrová, et al., 2000). However, enamel is also porous, with the inorganic component representing only 87% of the total volume. This fact means that approximately 13% of the volume of enamel is composed of organic material (matrix). This organic, protein-rich matrix is mainly water (11 percent of the total volume of enamel) (Harris & García-Godoy, 1999).

Apatite-like crystallites are highly organized hierarchical structures, and scanning electron microscopy (SEM) of enamel surfaces shows well-organized, rod-like apatite crystals, bundled in ordered prisms and elongated in their *c*-axis directions, which lie predominantly parallel to the rod axes. Despite these complex hierarchical structures, the basic building blocks for mineralized tissues are of nanoscale dimensions (Wang, et al., 2005; Pan, et al., 2008).

The hydroxyapatite crystals are oriented by a protein network that makes up the enamel matrix (Harris & García-Godoy, 1999). Amelogenin proteins constitute the primary structural entity of the extracellular protein framework of the developing enamel matrix (Wang, et al., 2005). Although enamel is not considered a living tissue because it has no cells or blood vessels, the overall protein network facilitates the diffusion of fluids, ions and small-sized molecules throughout the enamel (Harris & García-Godoy, 1999). The principal

elements found in enamel are Ca, P, sodium (Na), magnesium (Mg) and chlorine(Cl), and their mean concentrations are 37% Ca, 18% P, 0.4% Mg, 0.7% Na and 0.28% Cl (Reitznwrová, et al., 2000). Other elements reported to be consistently at or above the 1000-ppm level are Zinc (Zn) and silicon(Si), although some 40 other elements are known to be present in small and widely varying amounts (Eanes, 1979).

Ca and P are two biologically abundant elements, and P is an element shared by the collagen that directs biomineral precipitation. Apatite is a "sparingly soluble salt," making it a safe reservoir for Ca and P for use in other biological functions. Apatite's properties can be tailored to some extent through its ability to accept a wide range of chemical substitutions, each of which affects its chemical and physical properties. The most important property is apatite's extensive carbonate substitution, which controls lattice strain, solubility, the nature of substitution, and perhaps apatite's maximum crystal size (Pasteris, et al., 2008).

3. Incipient caries lesions around brackets

One common negative side effect of orthodontic treatment with fixed appliances is the development of incipient caries lesions around the brackets (Behnan, et al., 2010).

The demineralization of enamel adjacent to orthodontic brackets is a significant clinical problem. It has been reported that there is a significant increase in the prevalence and severity of enamel demineralization after orthodontic treatment when compared with untreated control subjects. The overall prevalence of white spot lesions among orthodontic patients has been reported as anywhere between 2 and 96 percent.

Once active orthodontic treatment has been completed, the demineralization process is normally expected to decelerate due to changes in local environmental factors. Some white spot lesions may remineralize and return either to normal or at least to a visually acceptable appearance. However, white spot lesions may also persist, resulting in aesthetically unacceptable results. In severe cases, restorative treatment may be required (Sudjalim, et al., 2006).

3.1 Etiology and development

It usually takes a period of months or even years for a carious lesion to develop; dental caries is not simply a continual, cumulative loss of mineral, but rather a dynamic process, characterized by alternating periods of demineralization and remineralization (Harris & García-Godoy, 1999). White spot lesions develop as a result of a dietary carbohydrate and saliva-modified bacterial infection (*Streptococcus mutans*), resulting in an imbalance between demineralization and remineralization of the enamel (Sudjalim, et al., 2006).

Demineralization is the dissolution of the calcium and phosphate ions from the hydroxyapatite crystals, which are lost into the plaque and saliva. In remineralization, calcium, phosphate, and other ions in the saliva and plaque are redeposited onto previously demineralized areas (Harris & García-Godoy, 1999). This is an interrupted process, with periods of remineralization and demineralization occurring (Sudjalim, et al., 2006). It is possible to have demineralization and remineralization occur without any loss of tooth mass (Harris & García-Godoy, 1999). However, depending on the state of the oral environment in terms of the prolonged accumulation and retention of bacterial plaque on the enamel surface, the standard of individual oral hygiene and the inherent resistance of that person (Sudjalim, et al., 2006), a lesion can result when the cumulative, negative mineral balance

exceeds the rate of remineralization over an extended period (Harris & García-Godoy 1999).

The incipient lesion is macroscopically evidenced by the appearance of an area of opacity – the so-called white spot lesion. During this stage, the carious process can be arrested or reversed (Harris & García-Godoy, 1999). A white spot lesion is the precursor of frank enamel caries (Sudjalim, et al., 2006). The most important fact is that the surface of the enamel is relatively intact, before any physical cavitation requiring clinical intervention has occurred (Harris & García-Godoy, 1999). The white appearance of early enamel caries is due to an optical phenomenon that is caused by mineral loss in the surface or subsurface enamel. Enamel crystal dissolution begins with subsurface demineralization, creating pores between the enamel rods. The resulting alteration of the refractive index in the affected area is a consequence of both surface roughness and loss of surface shine and of alterations in internal reflection, all resulting in greater visual enamel opacity, as porous enamel scatters more light than sound enamel. The demineralization process may encompass the full thickness of the enamel and some of the dentin before the relatively hypermineralized surface layer is actually lost.

It is generally accepted that the insertion of fixed orthodontic appliances creates stagnation areas for plaque and makes tooth cleaning more difficult. The irregular surfaces of brackets, bands, wires and other attachments also limit naturally occurring self-cleansing mechanisms, such as the movement of the oral musculature and saliva. This process, in turn, encourages a lower plaque pH in the presence of carbohydrates and accelerates the rate of plaque accumulation and plaque maturation (Sudjalim, et al., 2006).

The normal formation of a white spot lesion is usually a slower process, which can take 2 years or more. In other cases, such as seen in the xerostomia that follows head and neck radiation, lesions take at least 6 months to develop (Harris & García-Godoy, 1999). However, in patients under fixed orthodontic treatment, demineralized lesions have the potential to develop within 4 weeks of the initiation of the orthodontic treatment (Gorton & Featherstone, 2003; Staud, et al., 2004).

3.2 Prevention of white spot lesions

The development of white spot lesions during fixed-appliance orthodontic treatment can be prevented.

The chosen method or methods for prevention vary from patient to patient, depending on individual needs, available methods and the dentist's clinical criteria. However, the patient's motivation plays a very important role to be considered.

The available methods and techniques have focused primarily on patient education and topical fluoride administration, including the use of fluoride toothpaste, mouth rinses and gels, fluoride varnishes, fluoride in orthodontic bonding agents, fluoride in elastomeric modules and ligature ties. More recently, casein phosphopeptide-amorphous calcium phosphate and laser irradiation on the enamel surface have been introduced for preventive purposes (Schmit, 2002; Sudjalim, et al., 2006; Dun, 2007).

The aim of modern dentistry is the early prevention of tooth decay, rather than invasive restorative therapy (Hannig & Hannig, 2010; Cochrane, et al., 2010). However, despite tremendous efforts to promote oral hygiene and fluoridation, the prevention and biomimetic treatment of early caries lesions are still challenges for dental research and public health, particularly for individuals at high risk for developing caries, which is the most widespread oral disease (Hannig & Hannig, 2010).

The overall management of white spot lesions involves the consideration of methods of preventing desmineralization and also methods of encouraging the remineralization of existing lesions. Preventive measures take precedence, due to the challenging nature of treating patients who do develop significant numbers of white spot lesions. In addition to regular professional oral hygiene visits and the application of appropriate preventive medicaments, successful preventive strategies should involve oral health promotion, patient education and patient compliance (Sudjalim, et al., 2006).

4. Laser technology

4.1 General principles

The word “laser” is an acronym for light amplification by stimulated emission of radiation. Lasers have their basis in certain theories from the field of quantum mechanics, initially formulated during the early 1900s by the Danish physicist Niels Bohr, among others. Einstein’s atomic theories on controlled radiation can be credited as the foundation for laser technology. Einstein’s article on the stimulated emission of radiant energy, published in 1917, is acknowledged as the conceptual basis for amplified light (Sulewski, 2000).

A beam of light is composed of packets of photons, which are produced by a light bulb or other light sources (Stabholz, et al., 2003). The process of lasing occurs when an excited atom is stimulated to emit a photon before the process occurs spontaneously. Spontaneous emission of a photon by one atom stimulates the release of a subsequent photon and so on (Aoki, et al., 2004).

A laser beam implies stimulated emission of radiation and differs from conventional light source. It has a single wavelength (monochromatic) and is collimated (very low divergence), coherent (photons in phase), and intense. The construction of a light source, based on stimulated emission of radiation, requires an active medium, which is a collection of atoms or molecules. The active medium must be excited to emit the photons by stimulated emission; it may be a gas, liquid, or solid material and may be contained in a glass or ceramic tube (Stabholz, et al., 2003). The photon emitted has a specific wavelength that depends on the state of the electron’s energy when the photon is released. The characteristics of a laser depend on its wavelength (Aoki, et al., 2004). When tissue is irradiated, four basic types of laser interaction occur: reflection from tissue, scattering within tissue, absorption by tissue and transmission to the surrounding tissues (Aoki, et al., 2004; Stabholz et al., 2003). Transmission of light passes energy through the tissue without interaction and thus causes no effect or injury. When scattered, light travels in different directions, and energy is absorbed over a greater surface area, producing less intense and less precise thermal effects. Reflection results in little or no absorption and, subsequently, no thermal effect on the tissue. When absorbed by tissue, light energy is converted into thermal energy. A single laser device cannot perform all the required functions because the beam is absorbed or reflected according to its wavelength and the color of the object impacted (Stabholz, et al., 2003).

4.2 Laser technology development

The first laser device was created in 1960 by Maiman, and since then, the laser has been used in various areas of medicine, particularly in ophthalmology, otolaryngology, dermatology and surgery (Sulewski, 2000; Ishikawa et al., 2004).

In 1964, laser was introduced for use in dentistry by Stern, Sognnaes, and Goldman and by Hornby, Meyer and Goldman (Ishikawa et al., 2004). The first laboratory tests in vitro were carried out by Stern and Sognnaes and were limited to the use of ruby laser (Stern & Sognnaes, 1972). Then, due to technological advances, new lasers, such as argon ion, Nd:YAG, and CO₂ lasers, among others, were developed (Parker, 2007a). In time, other laser wavelengths, such as holmium (Ho):YAG and erbium (Er):YAG, were investigated.

Historically, the first lasers to be marketed for intraoral use were generally CO₂ lasers, with otorhinolaryngologic clearances authorized by the US Food and Drug Administration (FDA). During the 1970s and 1980s, intraoral use of CO₂ lasers was confined primarily to specialists, such as ear-nose-throat (ENT) surgeons, oral surgeons, and some periodontists.

It was not until 1990 that the field of laser dentistry began in earnest in the United States, at least in clinical terms. In May 1990, the FDA approved for intraoral soft tissue surgery a pulsed Nd:YAG laser, developed by Myers and Myers and recognized as the first laser designed specifically for general dentistry.

The use of lasers for therapy has become very common in the medical field, and the use of lasers in dentistry is extensive and covers many procedures, such as intraoral soft tissue surgery, hard tissue applications (e.g., caries removal, inhibition and detection, cavity preparation, etching, bleaching, calculus removal, bone ablation, cartilage reshaping, dentin desensitization, analgesia), composite curing, bracket debonding, allow welding, removal of canal debris, performing pulp capping, pulpotomy, and pulpectomy. Other dental applications include laser diagnostic, holography and biostimulation (Sulewski, et al., 2000; Dederich & Bushick, 2004).

4.3 Laser and caries prevention

In the 1960s, Stern et al. reported increased enamel acid resistance after ruby laser irradiation (Stern, et al., 1966). Since then, several studies on caries prevention have been published due to the development of several types of lasers, such as CO₂, (Stern & Sognnaes, 1972; Featherstone, et al., 1998; Hsu, et al., 2000; Tisai, et al., 2002; Kato, et al., 2003; Klein, et al., 2005; Steiner-Oliveira, et al., 2006), argon (Westerman, et al., 2002; Nammour, et al., 2005), Nd:YAG (Bahar & Tagomori, 1994; Hossain, et al., 2001a), and Diodo (Kato, et al., 2006) and more recently with the group of erbium lasers: Er, Cr:YSGG and Er:YAG (Kayano, et al., 1989; Fried, et al., 1996; Hossain, et al., 2001b; Apel, et al., 2004).

There are various theories to explain the reduced acid solubility of dental enamel after heating. For instance, the water permeability of dental enamel has been observed to be lower after heating. More hydroxide and pyrophosphate, but less carbonate, are also generally found, in comparison with unheated enamel (Apel, et al., 2002).

The most accepted theory regarding the mechanism by which laser irradiation enhances enamel acid resistance is the reduction of bound carbonate, when the enamel surface is heated to the range of 100–400°C (Holcom & Young, 1980; Fowler & Kuboda, 1986; Liu & Hsu, 2007). Nevertheless, the modification of organic matter has been reported as one of the mechanisms in laser-induced caries prevention (the organic blocking theory) (Ying, et al., 2004).

In laser therapy, several factors related to exposure need to be considered: the wavelength and energy density of the laser, the irradiation time, the focal distance, and water cooling

(Morioka, et al., 1991; Fried, et al., 1996; Featherstone, et al., 1998; Hossain, et al., 2000; Young, et al., 2000; Hsu, et al., 2000; Tisai, et al., 2002; Kato, et al., 2003; Matson, et al., 2002; Apel, et al., 2002, 2004, 2005; Rodrigues, et al., 2004; Westerman, et al., 2002; Nammour, et al., 2005; Cecchini, et al., 2005; Liu, et al., 2006; Fried, et al., 2006; Castelan, et al., 2007).

Furthermore, to achieve additional prevention, special attention has focused on laser irradiation, topical fluorides and remineralizing solution associations, showing very promising results (Hossain, et al., 2002; Delbem, et al., 2003; Chin-Ying, et al., 2004; Tepper et al., 2004; Rodrigues, et al., 2004; Kwon, et al., 2005; Ana, et al., 2006; Westerman, 2006; Tagliaferro et al., 2007).

4.4 Er:YAG laser

In 1975, Zharicov introduced the erbium-doped: yttrium-aluminum-garnet (Er:YAG) laser. The active medium of this laser is a solid crystal of yttrium-aluminum-garnet that is doped with erbium. This type of laser generates light with a wavelength of 2.94 μm (2.940 nm), in the near- and mid-infrared spectral range and close to the border-infrared and mid-infrared. Furthermore, it is highly absorbed by water because its wavelength coincides with the large absorption band for water. The absorption coefficient of water for the Er:YAG laser is theoretically 10 times higher than that of the CO₂ laser, i.e., 10.6 μm (10.600 nm), and 15.000 to 20.000 times higher than that of Nd:YAG (1.064 nm) (Ishikawa, et al., 2004).

The pulsed Er:YAG laser was approved in 1997 by the FDA for hard tissue treatment, such as caries removal and cavity preparation (Sulewski, 2000). Additionally, other intraoral applications, such as soft tissue surgery, sulcular debridement (1999) and osseous surgery (2004), have been permitted (Aoki, et al., 2004).

The Er:YAG is perhaps the most versatile of all the types of lasers available on the market today because of its many applications in dentistry for both hard and soft tissues. However, one of its limitations is its limited coagulative ability, compared with other types of lasers (Sulewski, 2000).

During Er:YAG laser irradiation, laser energy is absorbed selectively by water molecules and hydrous organic components of biological tissues, causing the evaporation of water and organic components and resulting in thermal effects due to the heat generated by this process (photothermal evaporation). Moreover, in hard tissue procedures, water vapor production induces an increase of internal pressure within the tissue, resulting in explosive expansion, called "microexplosion". These dynamic effects cause mechanical tissue collapse, resulting in "thermomechanical" or "photomechanical" ablation. This phenomenon has also been referred to as 'water-mediated explosive ablation' (Hibst & Keller, 1989; Keller & Hibst, 1989; Aoki, et al., 2004).

Although this laser was introduced into dentistry for the ablation of dental hard tissue (Hibst & Keller, 1989; Keller & Hibst, 1989), an early report (Kayano, et al., 1989) suggested an increase in acid resistance of the enamel adjacent to the ablated area. Later, several in vitro studies (Morioka, et al., 1991; Fried, et al., 1996; Hossain, et al., 2000; Cecchini, et al., 2005; Castelan, et al., 2007) on smooth dental enamel surfaces reported that Er:YAG irradiation promotes caries prevention as well as morphological enamel changes (Matson, et al., 2002; Cecchini, et al., 2005; Apel et al., 2005;). Furthermore, it was reported that both effects depend on the energy density of the laser, among other parameters, including

irradiation time, focal distance, and water cooling (Morioka, et al., 1991; Fried, et al., 1996; Hossain, et al., 2000; Young, et al., 2000; Matson, et al., 2002; Apel, et al; 2002, 2004, 2005; Cecchini, et al., 2005; Liu, et al., 2006; Castelan, et al., 2007).

4.5 CO₂ laser

In 1964, the CO₂ laser was developed at Bell Laboratories in the United States (Parker, 2007a). The active medium of CO₂ laser is a gas, and it emits infrared light at different wavelengths (9,300, 9,600, 10,300 and 10,600 nm) (Aoki, et al., 2004; Ishikawa, et al., 2004; Parker, 2007b). The 10,600 nm CO₂ laser is the most commercially available, and it is used as both a pulsed- and a continuous-wave laser (Rodrigues, et al., 2004). This laser is readily absorbed by water and therefore is very effective for surgery on soft tissues, which have high water content. The primary advantage of CO₂ laser surgery over the scalpel is its strong hemostatic and bactericidal effects. Very little wound contraction and minimal scarring are other advantages of laser surgery, especially for the CO₂ laser. However, the CO₂ laser is also highly absorbed by the principal mineral components of hard tissue, especially the phosphate ions ($- PO_4$) in carbonated hydroxyapatite. The energy applied is readily absorbed in hard tissues but causes instantaneous heat accumulation in the irradiated inorganic components, resulting in carbonization of the organic components and melting of the inorganic components, instead of the water-mediated physical collapse of hard tissues observed in Er:YAG laser irradiation (Aoki, et al., 2004). The caries-preventive effect produced on the enamel surface due to CO₂ laser irradiation was reported by Stern et al. (Stern, et al., 1972). Subsequently, several studies, both *in vitro* (Featherstone, et al. 1998; Kantorowitz, et al., 1998; Hsu, et al., 2000; Tsai, et al., 2002) and *in vivo* (Kato, et al., 2003), have shown increases in enamel acid resistance.

5. General-scope energy dispersive spectroscopy

Energy dispersive spectroscopy (EDS) is an analytical technique that allows the detection of the elements present in the studied material. It is very versatile and can be used with any type of solid sample, from metals and ceramics to biological tissues. It is usually coupled with an electron microscope to observe simultaneously the exact area of interest from which the signal will be collected.

One of the main advantages of EDS is its ability to detect almost all of the elements of the periodic table simultaneously and to quantify the amount of each of those elements present in a sample. Additionally, it is a very attractive technique because of the small amount of time that it takes to analyze a sample, which is typically on the order of minutes. However, when element mapping is needed, the measurements can take as long as several hours, depending on the material and the concentration of the elements of interest.

The main disadvantage of EDS is its inability to detect hydrogen (H), helium (He) and lithium (Li) due to the protecting window used in front of the detector that absorbs low-energy X-rays. This is an important disadvantage, especially for biological samples because many organic compounds and biomaterials are principally composed of carbon (C), oxygen (O) and hydrogen (H) (Williams, et al., 1996).

The main goal of this section is to show the way we use EDS coupled with a scanning electron microscope (SEM) in our research, which involves the chemical characterization of human teeth. For that purpose, we will begin with some fundamental aspects of the

technique that we have found are important to setting the analysis parameters properly and that help with understanding and interpreting the results.

5.1 X-ray production and fluorescence yield

When the incident electron beam reaches the sample, one phenomenon that occurs is that one electron of the inner shells of an atom is excited and ejected from the atom, leaving an electron hole. Another electron from an outer, higher energy shell fills the hole and in the process an X-ray is produced. As the energy of the X-ray is characteristic of the electronic structure of each element and of the differences in energy from one shell to another, this allows knowing from which element the X-ray came from by measuring its energy.

X-ray production is one of several de-excitation processes of an ionized atom. Therefore, it is important to have an idea of the amount of generated X-ray for each atom. The fluorescence yield w for the production of K shell radiation is given by the following equation:

$$\omega_K = \frac{\#K \text{ photons produced}}{\#K - \text{shell ionizations}}$$

The fluorescence yield is favored for high atomic number atoms. For example, the ω_K for carbon is approximately 0.005, while for germanium, it is approximately 0.5 and near unity for the heaviest atoms. The impact of this number on the analysis of biological samples is obvious: it takes many more beam electrons to produce a significant amount of X-rays from carbon (C), oxygen (O) or nitrogen (N) atoms. In practice, this fact means that a longer analysis time is required to obtain an adequate signal-to-noise ratio.

5.2 Interaction volume

Although it is said that SEM and coupled EDS are surface analytical techniques, this statement is not completely true. Due to the energy of their beam electrons, they are capable of penetrating a sample, interacting with its atoms and therefore generating signals (backscattered electrons, secondary electrons, characteristic X-rays, etc.). The space underneath the sample surface, where these signals are generated, is called the interaction volume (Goldstein, et al., 2003). The shape of the interaction volume varies from a hemisphere, for high atomic number atoms, to a pear, for low atomic number atoms, as seen in Fig. 1.

The dimensions and shape of the interaction volume depend on several factors, such as the energy of the electron beam, the average atomic number of the sample, the density of the material and the incidence angle of the beam.

The energy of the beam has a strong influence on the size of the interaction volume. For example, for iron, the interaction volume size ranges from 0.5 μm at 10 keV to 2 μm at 30 keV. This effect is much more pronounced in low atomic number materials; the interaction volume at 20 keV in a low atomic number target, such as a biopolymer, can be as large as several micrometers, depending on its density.

The next factor affecting the interaction volume is the atomic number Z . The larger the atom is, the higher the probability of interaction with the electron beam will be. Therefore, as Z increases, the interaction volume decreases. For example, at 20 keV, the interaction volume for carbon is approximately 8 μm , while for uranium, it is approximately 0.4 μm .

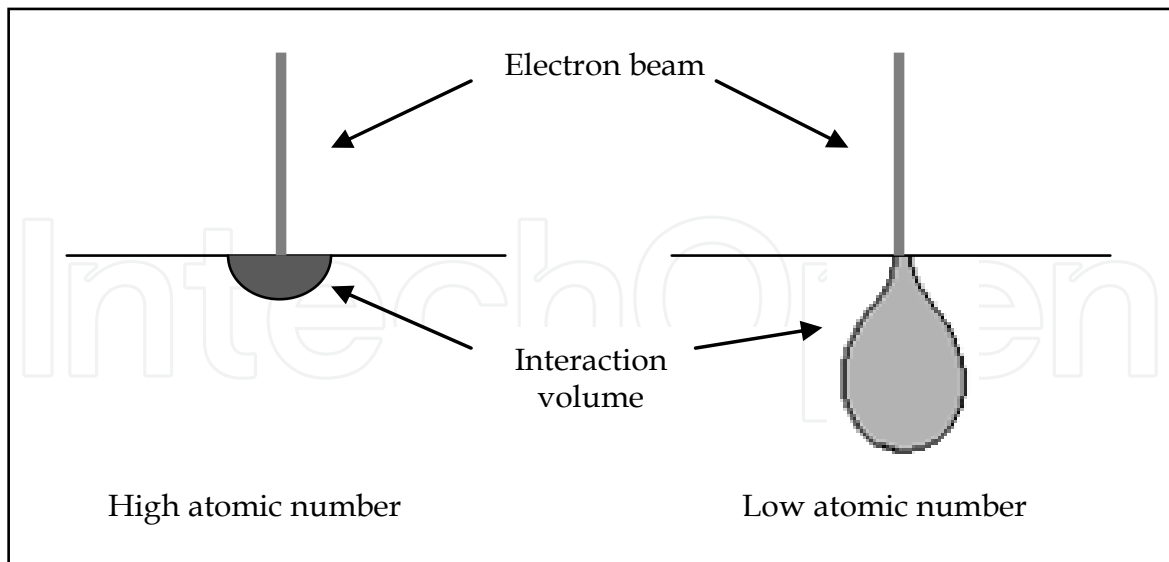


Fig. 1. Interaction volume from samples with different average atomic numbers

In dental research, it is very important to take into account these effects to avoid mistakes in the interpretation of the results. For example, during investigations in which the surface of the teeth are treated and low depths need to be studied, we recommend working with low-beam energy; otherwise, the volume underneath the treated surface will be analyzed together with the space of interest. However, as we decrease the beam energy, the number of generated X-rays also decreases. Thus, care should be taken when choosing the analysis parameters.

5.3 Qualitative analysis

EDS allows us to determine qualitatively the elements present in the specimen. This process is undertaken by analyzing the EDS spectrum and assigning an element to each peak on that spectrum. Today, modern equipment can perform this assignment automatically, although further analysis, done by the researcher, is necessary because many peaks from different elements overlap with the same energies. This fact is most important when analyzing samples, the composition of which is completely unknown.

5.4 Quantitative and semiquantitative analysis

In addition to qualitative analysis, EDS is widely used because of its ability to measure the concentrations of all elements present in samples (except for H, He and Li).. This process is undertaken by a series of measurements, for which the peak intensity from every element is compared with the peak intensity from a reference standard. The minimum detection limit of an EDS analyzer is approximately 0.1% for elements with $Z > 10$ and approximately 1.0 wt. % (weight percentage) for lighter elements (Brundle, et al., 1992).

To perform a true quantitative analysis, several issues must be taken into account. First, the sample and the reference must be flat, such that the surface imperfections do not interfere with the generated X-rays. Second, all the analysis parameters (e.g., accelerating voltage, spot size, time of analysis, etc.) must be the same. Additionally, the reference sample must contain the same elements as the studied specimen.

In practice, it is very difficult to meet all the requirements for a true quantitative analysis for several reasons: our samples are not always flat and free of surface defects, and we do not have a reference standard for each type of sample used (metallic, polymeric, biological, etc.). In such cases, the peaks from the elements in the specimen are compared with the peak of a pure element, usually copper. Some corrections should be made to have reliable results: an atomic number correction, accounting for the fraction of backscattered electrons by the sample within the interaction volume (the Z factor), a correction for the absorption of X-rays inside the sample (the A factor), and a correction for secondary X-ray fluorescence in the sample (the F factor). The most common procedure to take into account these factors is known as the ZAF correction.

In the case of a true quantitative analysis, the expected error is approximately 2% for major concentrations. When using a pure reference element, the error should be expected to be 4-5%. For elements with concentrations of less than 5% the relative error would be approximately 10%. This is why, when using a pure reference element, the process is often called a semiquantitative analysis.

In dental research, due to the inherent changes in morphology and composition of teeth from human to human and from tooth to tooth, it is virtually impossible to carry out a true quantitative analysis. However, a semiquantitative analysis is possible if we are careful during the analysis. The setup parameters of the microscope must always be the same (accelerating voltage, spot size, working distance, etc.). Since we deal with low atomic numbers (mainly O, Ca and P) the spectrum acquisition time should be long in order to have a good signal-to-noise ratio with well-defined peaks and a high number of counts. Calibration with the reference element should be performed every day or even every several hours to account for changes in the electronics of the system due to changes in the room temperature, vacuum, etc. Even with these precautions, as stated previously, we should consider a relative error in the calculations of approximately 10% or more if the concentration of certain elements is less than 5%.

Usually, in the revised literature, people working with teeth have tended to report the concentrations of elements using wt.%, perhaps as a convention. However, we have proposed the use of atomic percentage (at.%) instead, because it is more convenient to consider the difference in the number of atoms, rather than their weight. In this way, we can better correlate any changes in tooth composition directly with the molecular or atomic structure. Therefore, all of our results for tooth composition are presented in atomic percentages. Of course, one can change from one percentage to the other by relating each element composition to its corresponding atomic number.

6. Experimental design

The study protocol was reviewed and approved by the Research and Ethics Committee at the Autonomous University of the State of Mexico. All subjects enrolled in this research signed an informed consent.

6.1 Tooth selection

A total of 48 premolar teeth, extracted for orthodontic reasons, were stored in 0.1% (wt./vol) thymol solution at 4°C, until the experiment began. The teeth were selected according to the following criteria: intact buccal enamel surfaces without developmental defects,

restorations or fluorosis, not subjected to any pretreatment chemical agents, such as hydrogen peroxide, no cracks and no caries, according to the DIAGNOdent criteria (DIAGNOdent pen, KaVo, Biederach, Germany).

6.2 Sample preparation

All selected teeth were cleaned with a brush and deionized water; subsequently, they were dried with compressed, oil-free air. In the middle third of each root, a mesio-distal perforation was made, and then a fixture of stainless wire was introduced to attach each sample with self-curing acrylic to the top of a polyethylene container. For etching, a 35 percent phosphoric acid gel (Ultra-Etch; Ultradent, UT, USA) was used (15 sec). Then, the teeth were rinsed and dried again, as previously described. On the buccal surface, an orthodontic bracket (slot 0.018 inch standard edgewise premolar bracket, Ormco, CA, USA) was bonded to the enamel with a composite resin (Transbond XT cure, 3 M Unitek, CA, USA, Lot), following the manufacturer's instructions. The position of the orthodontic bracket was parallel to the longitudinal axis of the tooth, at a height of 4.0 mm, using bracket height gauges (Ortho™ Ormco, California, USA)..

6.3 Treatment of the enamel surface

The samples were randomly divided into four groups (n=12): Group 1 no laser irradiation (control group); Group 2 was treated with CO₂ laser; Group 3 was irradiated with Er:YAG laser; and Group 4 was treated with Er:YAG + remineralizing solution (Fig. 2). Each sample was irradiated twice during a time period of 18 sec.

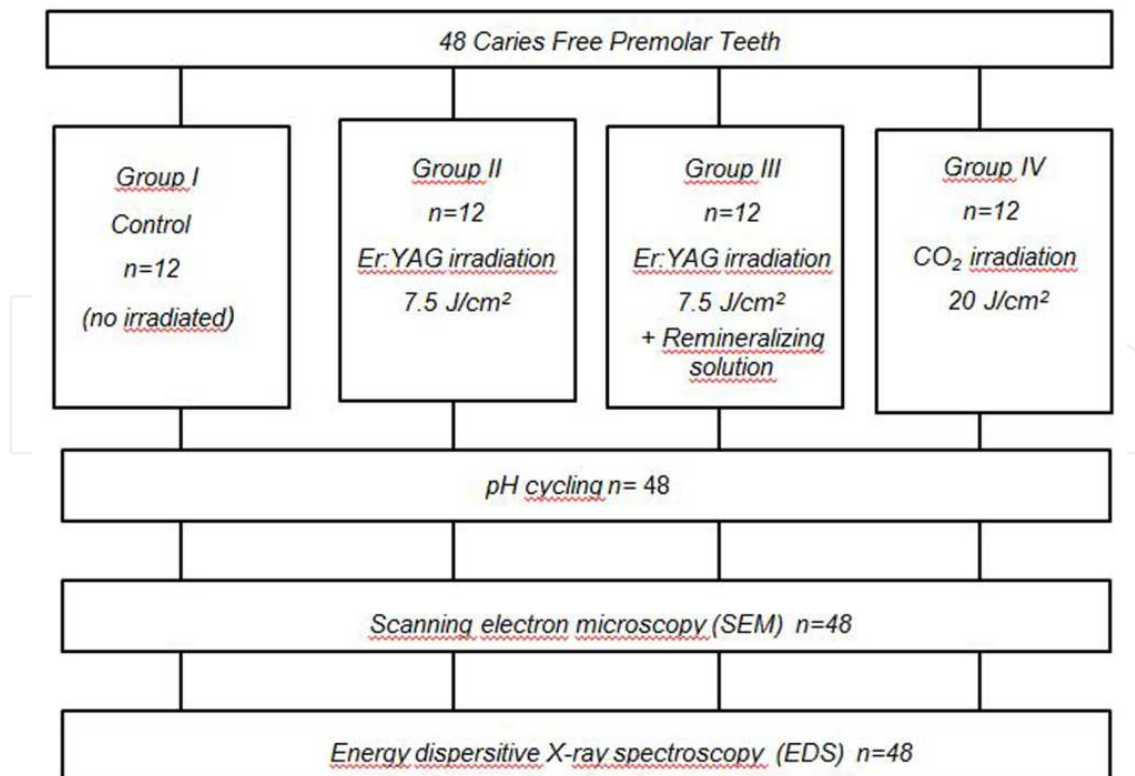


Fig. 2. Experimental design

6.4 pH cycling

After laser irradiation under specific conditions, a pH-cycling model of ten Cate and Duijster, modified by Featherstone (Featherstone, et al., 1998), was used to produce carious lesions, with a demineralization and remineralization period alternating daily over 9 days.

6.5 SEM and EDS

At the conclusion of the pH-cycling process, each tooth was rinsed with deionized water. Finally, the teeth were dried at room temperature and attached to a testing ring, using adhesive carbon paper (SPI Supplies, USA) to be observed by scanning electron microscopy (JEOL, JSM-6510LV, Japan). The atomic percentages (at.%) of calcium (Ca), phosphorus (P), carbon (C) and oxygen (O) on the enamel surface adjacent to the bracket were evaluated by energy dispersive spectroscopy (EDS) (Oxford Instrument, 7582, United Kingdom).

6.6 Statistical analysis

All data were analyzed using the SPSS 13.0 statistical package for Windows (SPSS Inc., Chicago, IL, USA). The measurements were analyzed using the Kruskal-Wallis and Mann-Whitney tests at a $p \leq 0.05$ level of significance.

7. Results

The most significant images obtained for each group are shown. The control group (not irradiated) displayed open enamel prisms (Fig. 3a). In the irradiated samples (Figs. 3b, 3c, 3d), rough areas, craters and cracks were observed.

The experimental data obtained by EDS for all groups regarding the percentages of Ca, P, C and O are displayed in Table 1. A similar content for Ca was observed in all groups; however, additional variations in the content of other elements, especially C, were found in Groups III and IV.

Groups	n	C	O	P	Ca
Group I	12	23.2 A ± 3.5	52.6 A ± 3.2	10.1 A ± 0.9	14.1 A ± 2.0
Group II	12	23.4 A ± 5.4	53.5 A ± 5.1	9.4 A ± 1.1	13.7 A ± 1.7
Group III	12	16.3 B ± 4.9	58.6 B ± 5.2	10.6 A ± 1.2	14.6 A ± 2.6
Group IV	12	14.5 C ± 6.5	57.3 A ± 6.7	11.5 B ± 1.5	16.7 A ± 3.9

*Groups with different letters are significantly different ($p \leq 0.05$), based on statistical analysis by element.

Table 1. Mean Values and Standard Deviations of Atomic Percentages for Each Element

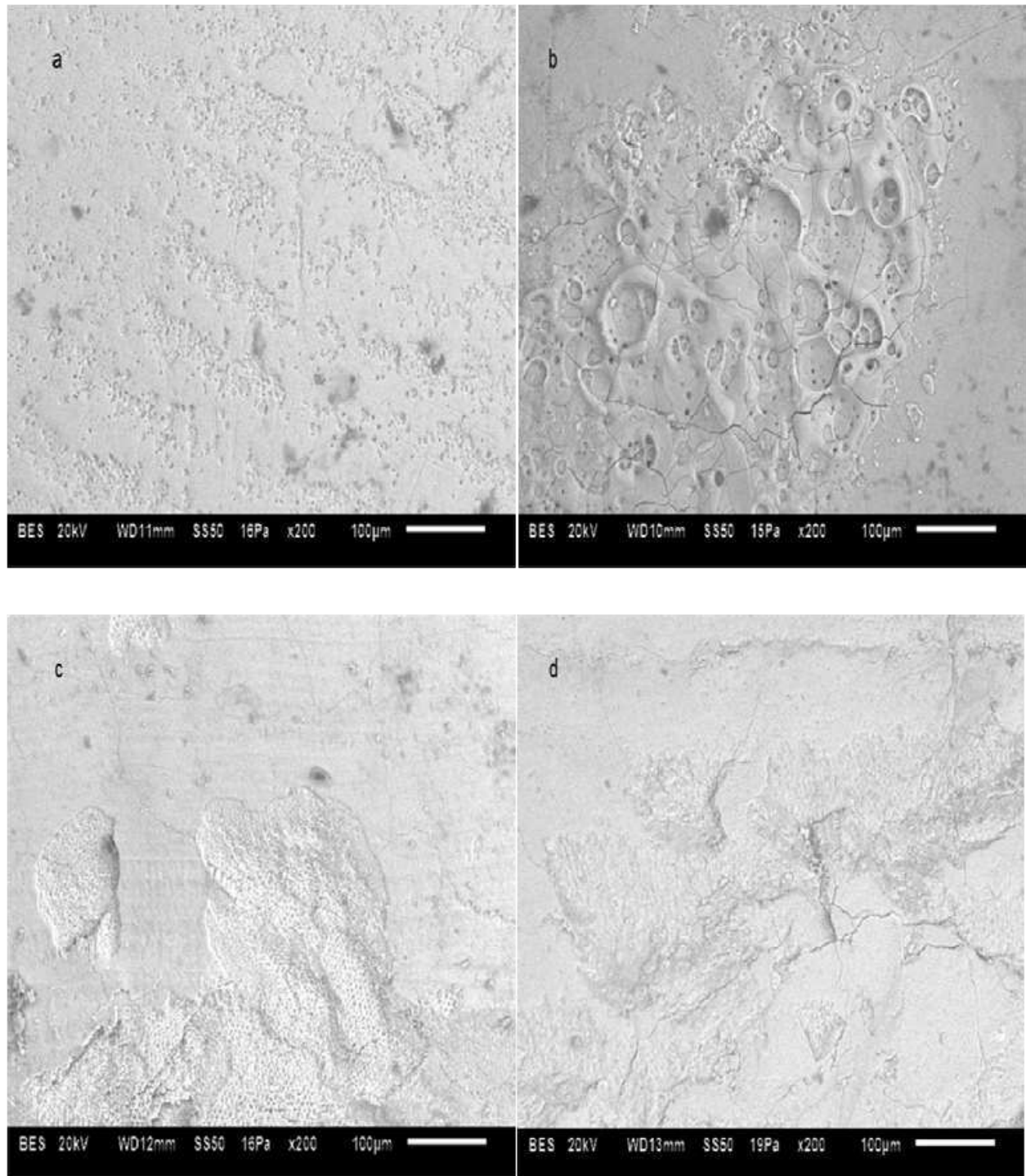


Fig. 3. Representative SEM micrographs of samples from the control group (a) and from experimental groups (b,c,d), showing the typical pattern of a non-irradiated enamel surface with exposed prisms (a), craters and melting produced by CO₂ laser irradiation (b) and craters produced by Er:YAG laser irradiation and evident demineralization due to a pH cycling process (c); also, craters and less demineralization are observed in Group IV (d). Original magnification x 200; scale bar =100 µm

8. Conclusion

The treatment conditions used on dental enamel surfaces for Groups II and IV likely induced changes in the structure of these biological tissues, which could interfere with the development of early lesions of dental caries. However, additional studies are required.

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Geriatric dentistry, or gerodontics, is the branch of dental care dealing with older adults involving the diagnosis, prevention, and treatment of problems associated with normal aging and age-related diseases as part of an interdisciplinary team with other healthcare professionals. Prosthodontics is the dental specialty pertaining to the diagnosis, treatment planning, rehabilitation, and maintenance of the oral function, comfort, appearance, and health of patients with clinical conditions associated with missing or deficient teeth and/or oral and maxillofacial tissues using biocompatible materials. Periodontology, or Periodontics, is the specialty of oral healthcare that concerns supporting structures of teeth, diseases, and conditions that affect them. The supporting tissues are known as the periodontium, which includes the gingiva (gums), alveolar bone, cementum, and the periodontal ligament. Oral biology deals with the microbiota and their interaction within the oral region. Research in oral health and systemic conditions concerns the effect of various systemic conditions on the oral cavity and conversely helps to diagnose various systemic conditions.

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