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ORIGINAL ARTICLE

Human dental enamel evaluation after radiotherapy simulation and laminates debonding with Er,Cr:YSGG using SEM and EDS

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Abstract:

The pursuit of perfection makes younger people undergo aesthetic procedures without formal indication. However, young patients may be susceptible to a disease such as head and neck cancer which treatment can compromise the adhesion of these indirect materials. Here, we present an analyze, of the gamma radiation effects on crystallographic morphology of human dental enamel after laminate veneer debonding with Er,Cr:YSGG laser. Thus, human dental enamel samples were prepared and randomized into 2 groups (n=10): Laser Irradiation (L) and Gamma + Laser Irradiation (GL) group. Scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) were performed before bonding and after debonding using Er,Cr:YSGG. Only Gamma + Laser Irradiation group received a cumulative dose of 70 Gy gamma radiation used in head and neck cancer radiotherapy. SEM images showed that both GL and L groups presented altered morphology. EDS showed an decrease in Ca and P intensities after laser debonding of laminates veneers in both group. Therefore, a proper laser facet removal protocol should be established for healthy patients and patients who have been exposed to radiotherapy for head and neck cancer.

Keywords: Radiotherapy; Gamma Rays; Lasers, Solid-State; Dental Enamel; Head and Neck Neoplasms

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INTRODUCTION

Head and neck cancer are the sixth most common cancer in the world population and can be treated with radiotherapy, chemotherapy, surgery or combination of these procedures according localization and stage of disease. The head and neck cancer may affect the oral cavity, pharynx, larynx, nasal cavities and major and minor salivary glands[1]. The proper treatment of cancer is very important for patient healing. However, all treatment alternatives can present physical and psychological effects in patient.

The gamma radiation dose in head and neck cancer radiotherapy promote a biochemical alteration in buccal environment because the local effect of gamma radiation in salivary glands promoting hypo-salivation, xerostomia and decreased concentration of inorganic ions calcium and phosphate. Consequently, the buffering capacity of the saliva becomes less effective, and teeth are susceptible to demineralizing action of acids presenting radiation cavities, among other comorbidities into the mouth. In addition, gamma radiation causes dehydration and weakens the intermolecular bonds between enamel and dentin, making the tooth more susceptible to fracture[1-4].

Porcelain is an aesthetic material used in many types of indirect oral rehabilitation that present high wear resistance and translucency similar of human dental enamel. Aesthetic laminates are indicated in cases of diastema, tooth pigmentation among others. Indirect oral rehabilitation with ceramic materials, when properly indicated, promotes improved patient self-esteem and confidence in social relationships. However, even indirect materials needs to be replaced over the years[5,6]. Therefore, it is possible that biochemical and biomechanical changes in human dental enamel structure after gamma radiation compromise the maintenance of an indirect material.

MATERIALS AND METHODS

It was conducted an *in vitro* randomized study using 20 human dental enamels. The samples were divided in two groups: laser irradiation and gamma+laser irradiations. The chemical and morphological assessing was performed using Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDS), before and after the irradiations.

Samples preparation

After approval by the Ethics Committee of the Faculty of Dentistry from the University of São Paulo (CAAE 02717618.8.0000.0075), human dental enamel samples were prepared and randomized in 2 groups (n=10): Laser Irradiation (L) and Gamma + Laser Irradiations (GL). For that, tooth was previously decontaminated with a thymol solution[7], cut in Isomet® Precision Metallographic Cutter (Buehler, Chicago, United States of America), embedded in acrylic resin (VipiCril, São Paulo, Brazil) to facilitate manipulation, and polished in EcoMet® 250 (Buehler, Chicago, United States of America). Subsequently, to homogenize samples by Knoop hardness number similar to human dental enamel (~300 kg/mm^2), the surface microhardness were performed on microhardness tester (Shimadzu HMV-2000, Kyoto, Japan), and was selected the hardness number value used to human enamel test (245.2 mN/HK 0.025).

Laminates bonding

The adhesive used on the enamel surface was Tetric N Bond (Ivoclar Vivadent, Schaan, Liechtenstein) and the primer applied to laminate veneers was Monobond (Ivoclar Vivadent, Schaan, Liechtenstein). The resin cement used Variolink N® (Ivoclar Vivant, Schaan, Liechtenstein) to laminates bonding in human dental enamel samples.

Gamma Irradiation

The enamel samples with laminate were exposed to gamma radiation using a Cobalt-60 teletherapy unit (Theratron Phoenix External Beam Therapy System, Best Theratronics Ltd., Ottawa, Canada) from the multipurpose irradiator of IPEN. It was established a cumulative dose of 70 Gy to simulate an usual head and neck cancer radiotherapy clinical protocol[8].

Laser Irradiation

The laser irradiation for the laminates debonding was performed using an Er,Cr:YSGG laser (Waterlase; Biolase, Inc, San Clement, CA, USA), emitting approximately 2780 nm. Each sample was laser irradiated using laser fiber tip axial exchangeable, model/version MGG6 Sapphire (Biolase, Inc), with 400µm length and 600 µm diameter. The average laser power was 1.41 W at a repetition rate of 20 Hz, and pulse duration approximately 140µs (~25 J/cm^2), with settings for 40:60% (water:air). The samples were positioned in a stepper motor device

(Universal motion controller/Driver (Newport Corporation®, California, United States of America), controlling X-Y during the irradiation, and the laser handpiece was mounted in optical supports, at 1mm distance from sample, and 90º angulation with it.

SEM/EDS imaging

The morphology of the human dental enamel mineral matrix was investigated by SEM using a Hitachi TM3000 (Hitachi, Chiyoda, Tokyo, Japan), instrument equipped with an accessory for EDS analyses using Quantax 70 software (Bruker, Stuttgart, Germany) for Hitachi equipment. The images were obtained at an acceleration voltage of 15kV at a magnification of x2000 on the enamel surfaces without sample preparation. The percentage element contents of specimens were determined by EDS. To obtain the elements proportion, the specimens were analyzed before and after the experimental procedures for each group to determine the percentage increases of calcium (Ca) and phosphorus (P). The profile spectra and x-ray beam intensities have been determined on circular area of 4 to 5 µm diameter, according sample.

Statistical analysis

Statistical analysis was performed for EDS data. The distribution and normality of variances were tested using D'Agostino-Pearson and Shapiro-Wilk methods. The pre and post irradiations comparisons were performed using two-tailed Student's t-test. All tests considered a level of significance of 5%.

RESULTS

The most representative micrographs of enamel and debonding after laser and gamma+laser irradiations are shown in Figures 1 and 2, respectively. Enamel surface after irradiations shows similar changes on their surfaces. In the SEM images, enamel pitting erosion with secondary pitting inside cavities were visible over the specimens surfaces after irradiations. Some artefacts of laminate debonding were shown in Fig. 1.

The EDS spectra obtained, exhibit the bands of elements presents in enamel matrix, and others traces of elements (not shown). In the study, the EDS analysis measured the relative intensities of Ca and P in total element content. The intensities were area normalized, to avoid differences among total element content in weight and atomic per cent, due to different regions of interest chosen. The t-Student test (Figs. 3 and 4) indicated that there was a statistically significance reduction in Ca

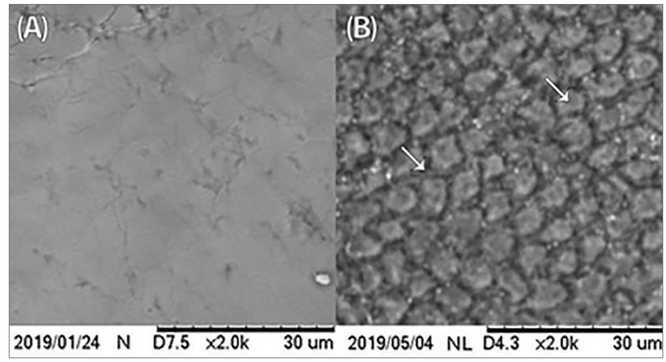

Figure 1. Representative micrographs of gamma + laser group. (A) enamel before bonding; (B) after debonding. Arrows indicate pitting erosion areas.

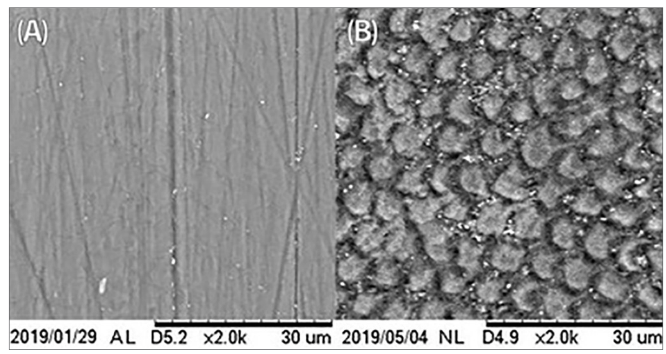

Figure 2. Representative micrographs of laser group. (A) enamel before bonding; (B) after debonding.

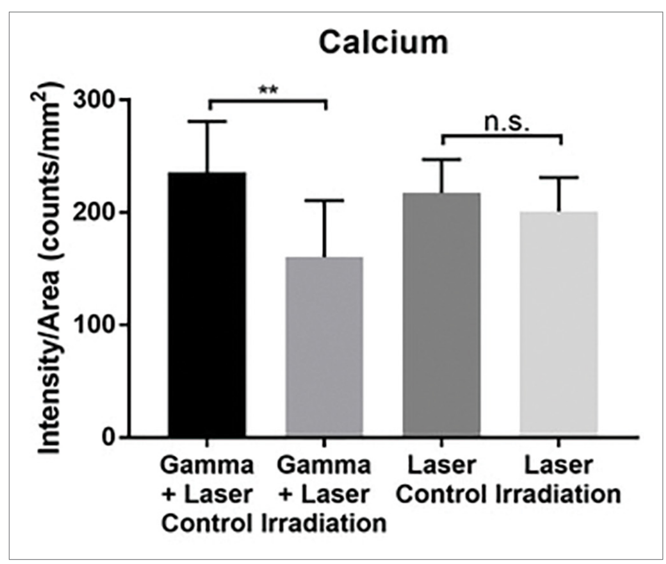

Figure 3. Student's t-test for calcium analysis. Stars denote significant statistical difference level.

and P, among groups control and gamma+laser irradiated. The specimens results in the group control and laser irradiated, shown not statistical significance. No significant difference in Ca/P ratio was detected among the groups control and irradiated (Fig. 5).

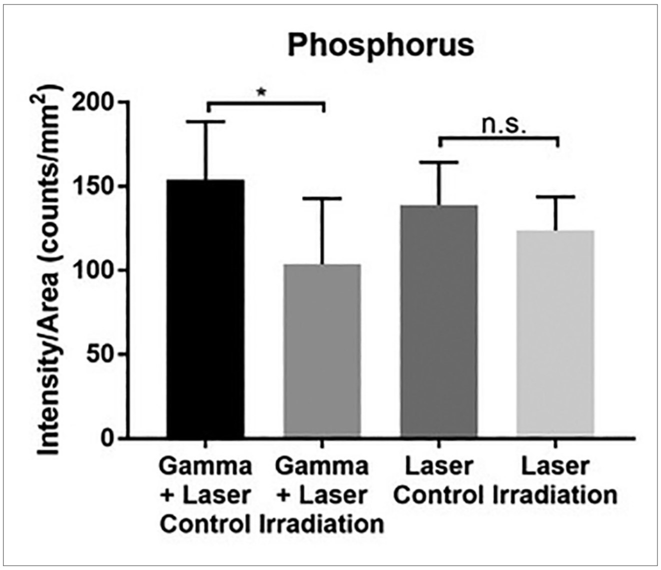

Figure 4. Student's t-test for phosphorus analysis. Stars denote significant statistical difference level.

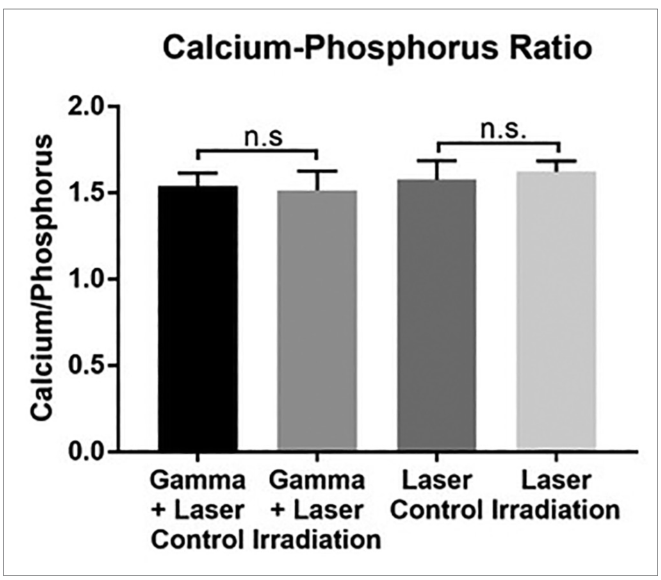

Figure 5. Student's *t*-test for calcium-phosphorus ratio analysis.

DISCUSSION

Radiotherapy for head and neck cancer (RHNC) is a treatment that causes much oral comorbidity such as mucositis, xerostomia, hypo-salivation and radiation cavities, compromising the systemic health of the patient. This important set of comorbidities may also interfere with the course of treatment as it may compromise the overall health of the patient leading to infectious conditions and malnutrition[9,10].

Cavities related to radiation exposure affects 25% of patients exposed to gamma radiation during RHNC and develops in a shorter period than sucrose-dependent caries that occurs due to interaction of etiological factors[11]. The cavities related to radiation exposure occur due to biochemical alteration in buccal environment, mainly the decrease of salivary capacity. Consequently, the gamma radiation in dental tissues can compromise the maintenance of the aesthetic indirect restorative treatment.

Clinical research indicates that hypo-salivation in buccal environment during gamma radiation alters the biochemical composition of human dental enamel and makes it difficult to replace restorative adhesive materials such as resin, glass ionomer and resin modified ionomer[12]. Some *in vitro* simulation of the head and neck radiotherapy studies indicate that both adhesive system and resin restorative materials can be compromised. It can occur after gamma radiation exposure mainly when aesthetical rehabilitation is in place[13].

The SEM images of Laser Irradiation group, after laser laminates removal, demonstrated alteration of prismatic structure of human enamel, mainly due to photoablative effects of laser in hydroxyapatite and water molecules (Fig. 1). Gamma+Laser Irradiation shown alterations in the prismatic conformation after laminates debonding, due to pitting erosion. This fact can be related not only to the photoablation of laser in enamel hydroxyapatite and water molecules, but also due to water radiolysis caused by gamma radiation[14,15].

After laminates debonding with Er,Cr:YSGG laser, it was observed no significant statistical difference in calcium and phosphorus quantitative analysis. This finding indicates that the laser irradiation did not promoted changes in enamel. Nevertheless, there was a significant decrease of both calcium and phosphorus after laser debonding in gamma irradiated samples, pointing that gamma-irradiated samples respond differently to laser laminate debonding. Calcium and phosphorus are two of the main constituents of hydroxyapatite, and its decrease in enamel content resulted in decreased mechanical properties[16].

In the evaluation of the Ca/P ratio, no significant difference was observed. Fanovich et al.[17] and Ślósarczyk et al.[18] reported that the Ca/P ratio is considered an important parameter as both the mechanical properties and biodegradation rate strongly depend on it. In the surface of enamel the mean Ca/P ratio of groups gamma+laser control and gamma+laser irradiation were 1.53 and 1.51, indicating a proportional decrease of Ca and P. For the group laser control and laser irradiation the mean Ca/P ratio were 1.57 and 1.62, indicating that values of Ca and

P does not decreased proportionally. These findings does not indicate that the chemical structure of the superficial layers of enamel was modified, however further studies will be needed to evaluate this condition.

The microstructural changes after gamma radiation and Er,Cr:YSGG laser exposition may compromise enamel recondition for future porcelain laminate veneer exchange. Moreover, the protocol for laminates removal in healthy patients should be different from the protocol used for patients exposed to gamma radiation during a radiotherapy treatment of head and neck cancer, since this kind of treatment may change the enamel chemical composition.

CONCLUSION

Gamma and Er,Cr:YSGG laser irradiations change the morphological aspects of enamel samples, which may compromise future laminate exchange. The SEM micrograph shows pitting erosion at enamel surface and the EDS analysis showed a decrease in Ca and P, which may be indicative of biodegradation, although Ca/P ratio did not show statistical difference. When performed before the laser laminate debonding, the radiotherapy simulation, using head and neck cancer treatment dose, compromised the enamel mineral content, where this fact should be considered for clinical applications.

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