Archives of Oral Biology 80 (2017) 130–135

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/archoralbio

Research Paper

Effects of different radiation doses on the microhardness, superficial morphology, and mineral components of human enamel

Sandra Ribeiro de Barros da Cunha[a], Felipe Paiva Fonseca[b], Pedro Augusto Minorin Mendes Ramos[c], Cecília Maria Kalil Haddad[c], Eduardo Rodrigues Fregnani[d], Ana Cecília Corrêa Aranha[e,*]

[a] Department of Restorative Dentistry, School of Dentistry, University of São Paulo, Av. Professor Lineu Prestes, 2227, São Paulo, SP, Brazil
[b] Department of Oral Surgery and Pathology, School of Dentistry,Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, Pampulha, Belo Horizonte, MG, Brazil
[c] Department of Medical Physicist, Hospital Sírio Libanês, Rua Dona Adma Jafet, 91 – Bela Vista, São Paulo, SP, Brazil
[d] Department of Oral Medicine, Hospital Sírio Libanês, Rua Dona Adma Jafet, 91 – Bela Vista, São Paulo, SP, Brazil
[e] Department of Restorative Dentistry, School of Dentistry, University of São Paulo, Av. Professor Lineu Prestes, 2227, São Paulo, SP, Brazil

ARTICLE INFO

Keywords:
Radiotherapy
Enamel
Microhardness
EDS
SEM
Head and neck cancer

ABSTRACT

Objective: To evaluate the effects of three different radiotherapy doses (20, 40, and 70 Gy) on the microhardness, superficial morphology, and mineral content (based on Ca and P values) of three different depths of human enamel (cervical, middle, and occlusal).
Design: Thirty-four third molars were cut, separated, and prepared. Microhardness samples (n = 30) were embedded in acrylic resin and then polished, and depths were delimited. Microhardness tests were performed on cervical, middle, and occlusal enamel pre- and post-radiotherapy with a load of 50 g for 30 s. For the scanning electron microscopy (SEM) analysis (n = 4) and energy dispersive X-ray spectroscopy (EDS) (n = 12), samples were fixed in a 3% glutaraldehyde solution, washed in 0.1 M cacodylate solution, and dehydrated in crescent concentrations of ethanol. Microhardness data were tested for significant differences using a two-way analysis of variance (ANOVA) and Tukey's test ($p < 0.05$), while SEM and EDS were evaluated qualitatively.
Results: The results showed a decrease in microhardness values only in the cervical enamel, regardless of the radiation dose used; no morphological or mineral change was observed.
Conclusion: Radiotherapy can affect the microhardness values of only cervical enamel without compromising the morphological or mineral (Ca and P) content at any depth.

1. Introduction

Head and neck cancer represents a heterogeneous group of malignant tumors of the upper aerodigestive tract. This type of cancer is the seventh most common worldwide (Jemal, Bray, Center, Ferlay, & Ward, 2011).

Oral cancer, which includes oral mucosa, hard and soft palate, gums, tongue, and floor of the mouth, is considered a worldwide health problem. Oral squamous cell carcinoma is the most common microscopic subtype, accounting for over 90% of all cases diagnosed. It is mainly associated with the simultaneous use of tobacco and alcohol, and in the year 2012 approximately 300,000 new cases and 145,000 deaths were estimated to have occurred worldwide from this type of cancer (Döbrossy, 2005).

During the last decades, there have been improvements in head and neck cancer treatment, and radiotherapy is an important part (El-Faramawy, Ameen, El-Haddad, & El-Zainy, 2013). According to the World Health Organization (WHO), two-thirds of oncological patients will have radiotherapy as a treatment option.

Radiotherapy is a local treatment that uses electromagnetic waves that carry energy and have no mass. The electromagnetic waves used in radiotherapy treatment are considered ionizing radiation, which means that the energy carried by these waves is higher than the energy that holds the electron in the electrosphere, creating free electrons. These free electrons can cause damage to the cell both directly and indirectly (Tauhata, Salati, Di Prinzio, & Di Prinzio, 2014). Indirect damage is caused by the production of free radicals from the radiolysis of different molecules, such as water that produces H^+ and OH^- ions, and the latter are highly unstable and reactive. These ions will bind to other molecules, which will be damaged and lose function, or will produce

* Corresponding author.
E-mail addresses: sandra.cunha@gmail.com (S.R. de Barros da Cunha), acca@usp.br (A.C.C. Aranha).

http://dx.doi.org/10.1016/j.archoralbio.2017.04.007
Received 20 February 2017; Received in revised form 5 April 2017; Accepted 8 April 2017

more free radicals. This radiation mechanism supports the consensus in the literature that radiotherapy of dental tissues has greater deleterious effects when higher concentrations of organic content are present in the tissue (Pioch, Golfels, & Staehle, 1992; Soares et al., 2010; Walker, 1975).

The standard treatment for squamous cell carcinoma in the head and neck regions is a final accumulated dose between 40 Gy and 70 Gy, which is fractionated in daily doses (Jham & da Silva-Freire, 2006; Kielbassa, Hinkelbein, Hellwig, & Meyer-Lückel, 2006a; Kielbassa, Hellwig, & Meyer-Lueckel, 2006b; Lieshout & Bots, 2014; Nutting et al., 2001; Seikaly et al., 2004). Despite the advantage of preserving the tissue structure, radiotherapy in patients with head and neck cancer usually results in oral complications (Jham & da Silva-Freire, 2006; Pioch et al., 1992; Soares et al., 2010).

The literature reports changes in the salivary glands, decreased salivary flow, changes in salivary composition and oral microbiota, oral mucositis, muscular trismus, vascular alterations, and osteoradionecrosis from radiotherapy (Kielbassa et al., 2006; Lieshout & Bots, 2014; Naves, Novais, Armstrong, Correr-Sobrinho, & Soares, 2012; Nutting et al., 2001; Silva, Alves, Berger, Giannini, & Goes, 2010). In addition to these structural changes, there is also the possibility that radiotherapy may exert direct effects on the teeth, such as changes in the dentin-enamel junction, crystalline structure, acid solubility of enamel, dentin elastic modulus, matrix metalloproteinases (MMPs), and enamel and dentin microhardness (Gonçalves et al., 2014; Kielbassa et al., 2006a, 2006b; McGuire, Gorski, Dusevich, Wang, & Walker, 2014; Reed et al., 2015; Silva et al., 2010).

One of the most cited side effects of radiotherapy is radiation-related caries, which are a complex, multifactorial disease and an indirect effect of radiation treatment that harms the tooth structure. It is a rapid, painless, and destructive form of tooth decay that can quickly lead to the amputation of crowns (Dreizen, Daly, Drane, & Brown, 1977; Vissink, Jansma, Spijkervet, Burlage, & Coppes, 2003).

With the increasing life expectancy of the population worldwide, longevity of the teeth in the oral cavity, and increase in neoplastic diseases, it is essential that dentists be aware of changes in the oral cavity produced by the irradiation of head and neck cancer so that they can manage patients correctly both pre- and post-treatment.

Thus, this study aimed to analyze superficial morphology, microhardness, and mineral content alterations of irradiated dental substrates at three different radiation doses.

2. Material and methods

2.1. Specimen preparation

Thirty-four healthy human third molars were collected from the Tooth Bank of the School of Dentistry of the University of São Paulo, after approval from the Research Ethics Committee of the same institution (protocol 658.991). Teeth were cleaned and had their roots removed with a water-cooled diamond disc (Kg Sorensen, Barueri, São Paulo, Brazil). From each tooth, two sagittal slices were obtained with a low-speed diamond saw under water cooling (Isomet 1000, Buehler Ltd., Lake Bluff, IL, USA). Sagittal sections were prepared at the middle of the crown along the long axis of the tooth creating two slices: the mesial and distal slices. The mesial parts were used for microhardness and energy dispersive X-ray spectroscopy (EDS), and the three distal parts were used for the EDS control group. The remaining four teeth were cut along a transverse plane to obtain three samples for each depth of enamel and used for scanning electron microscopy (SEM) (Fluxogram 1).

2.2. Microhardness

Microhardness refers to the testing of different materials hardness by applying small loads to it. The hardness of oral tissues is an essential aspect of their behavior, representing their resistance to masticatory loads, changes in the dentin-enamel junction, crystalline structure, acid solubility of enamel and dentin elastic modulus.

For the microhardness test, samples were embedded in acrylic resin, and then a decreasing sequence of silicon carbide (SiC) paper (400, 600, 800, 1200, 2500, and 4000 grit) was used with the aid of a water-cooled polishing machine (Buehler Ltd., Lake Buff, IL, USA) to obtain a smooth and polished surface. The opposite surface of each specimen was also polished to obtain a parallel surface, which is essential for the correct measurement of microhardness. Between each polishing stage, samples were cleaned using distilled water in an ultrasonic cleaner (Digital Ultrasonic Cleaner CD-4820, Kondortech, Sao Carlos, Brazil) for 8 min to remove any debris.

The enamel depths were delineated with a permanent marker and ruler. Five indentations at each enamel depth were performed with a Knoop tip attached to a Microhardness Tester HMV-2000 (SHIMADZU Co., Tokyo, Japan) using a load of 50 g for 30 s. The indentations started 150 µm away from the dentin-enamel junction, and each indentation was spaced 100 µm apart. Microhardness values were measured with CAMS-WIN software (Newage Testing Instruments Inc., Southampton, England).

The microhardness test was performed before and after irradiation so that each sample could serve as its own control.

After the first microhardness test analysis (control), the samples were randomly divided into three groups (n = 10) according to the radiation dose (Group 20 Gy, Group 40 Gy, and Group 70 Gy) and again submitted to the same microhardness analysis protocol.

The results were separated according to depth. The quantitative analysis was performed by a two-way analysis of variance (ANOVA) (doses of 20, 40, and 70 Gy before and after radiotherapy) with one repeated measurement (time) and at a significance level of 5% ($p \geq 0,05$).

2.3. Irradiation of the samples

Samples were irradiated with a total and single application of 20, 40, or 70 Gy with X-rays from a linear accelerator (Mevatron MX2 6 mV; Siemens Healthcare, Erlangen, Germany) in the Department of Radiotherapy at Sírio-Libanês Hospital. This study used a 20-Gy dose in an attempt to simulate teeth that are situated in healthy tissues, but located near the target region, which can receive between 40 and 70-Gy. Samples were submerged in distilled water during radiotherapy.

Moreover, all irradiations were done in the same container with the samples submerged in distilled water. An isocentric set-up was done, so that the geometric center of the container was aligned with the isocenter of the linear accelerator. This means that from whatever angle we point the gantry, the distance between the “radiation source” and the container's center would be 100 cm. For a homogeneous radiation distribution for all samples, radiation was applied in two opposing fields parallel to the incident radiation.

2.4. Energy dispersive X-ray spectroscopy

For the EDS test, 12 samples (n = 3) randomly selected from the microhardness analysis were used from the control and irradiated groups (20 Gy, 40 Gy, and 70 Gy).

For the control group, the distal part of the tooth that was not used for SEM was selected. For samples used in the microhardness analysis, the acrylic resin was removed with the aid of a low-speed cutting machine (Labcut 1010 − Extec, Enfield, Connecticut, EUA). Samples were then fixed in 2.5% glutaraldehyde solution for 24 h at 4 °C, washed in a solution of 0.1 M cacodylate, dehydrated in sequential ethanol baths for 10 min each (30%, 50%, 70%, 90%, 96%, and 100% for 20 min) and then transferred to a critical-point dryer (HMDS) for 30 min. Samples were mounted on aluminum stubs, properly identified, covered with a thin layer of carbon (MED 010, Balzers Union, Balzers,