Radiotherapy compositional and mechanical effects evaluation in radicular dentin by FTIR spectroscopy and microhardness testing

Daniela C. Figueredo
Center of Engineering, Modeling and Applied Social Sciences
Federal University of ABC
São Bernardo do Campo, Brazil
danielacfigueredo@gmail.com

Matheus del Valle
Lasers and Applications Center
Nuclear and Energy Research Institute
São Paulo, Brazil
matheus.valle@usp.br

Denise M. Zezell
Lasers and Applications Center
Nuclear and Energy Research Institute
São Paulo, Brazil
zezell@usp.br

Juliana K. M. B. Daguano
Center of Engineering, Modeling and Applied Social Sciences
Federal University of ABC
São Bernardo do Campo, Brazil
juliana.daguano@ufabc.edu.br>

Patricia A. Ana
Center of Engineering, Modeling and Applied Social Sciences
Federal University of ABC
São Bernardo do Campo, Brazil
patricia.ana@ufabc.edu.br

***Abstract*— Head and neck cancers are responsible for 400 to 600 thousand of new cancer cases per year over the world, presenting a mortality rate of 223 to 300 thousand per year. Radiotherapy can be used as a definitive, adjuvant or palliative treatment. One of the radiotherapy indirect side effect is the radiation-related caries, characterized by presenting a painless, rampant and rapid progression. Hard tissues can be affected by the ionizing radiation, changing their composition, morphology and mechanical properties, although there is a lack of studies relating these changes with radiation caries. Thus, considering that radicular dentin is a tissue with a high incidence of radiation caries, this study aimed to evaluate the compositional and mechanical changes of this tissue when submitted to radiotherapy. To that, it was conducted an in vitro study with 45 bovine teeth roots, where 90 radicular dentin slabs of 4 x 4 cm were obtained. The samples were immersed in artificial saliva and submitted to radiotherapy using cobalt-60, simulating doses for head and neck cancers of 2 Gy per day per 6 weeks, accumulating a total of 60 Gy. FTIR spectroscopy and microhardness testing were performed before and after the radiotherapy. The spectra were analyzed in six IR fingerprint bands: amide I, amide II, amide III, phosphate, carbonate ν_2 and carbonate $\nu_3\nu_4$. Statistical analysis was performed by Student's t-test and Wilcoxon test, considering the level of significance of 5%. The carbonate $\nu_3\nu_4$ and the amide III areas decreased after the radiotherapy, presenting significant statistical difference between both periods. These findings can be related to inorganic and organic changes due to free radicals formation. The Knoop microhardness value decreased after the radiotherapy, also showing statistical difference, which can be associated to collagen reorganization or denaturation and to inorganic changes, leading the tissue to be more prone to cracks formation. It can be concluded that the radiotherapy affects the compositional and mechanical properties of the radicular dentin, and this fact should be considered in future studies.**

Keywords—radiotherapy, radiation caries, FTIR, microhardness, dentin.

I. Introduction

Head and neck cancer comprise malignant tumors that affect the oral cavity, pharynx, larynx, paranasal sinuses, nasal cavity and salivary glands [1, 2]. This cancer type is responsible for 400 to 600 thousand of new cancer cases per year over the world, presenting a mortality rate of 223 to 300 thousand per year [3].

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The prognosis depends on a multifactorial analysis of the tumor location and staging, histopathological characteristics, comorbidities existence, and patient's age [1, 2, 4]. Clinical treatments can be performed by one or more procedures in combination, including surgery, radiotherapy, chemotherapy and immunotherapy [1, 2, 5].

Radiotherapy is a technique based on free radicals formatting due to the ionizing radiation interaction with tissue water. Free radicals can cause direct or indirect damage to the cells' DNA, thus preventing the replication of neoplastic cells [4]. This technique can be used as a definitive, adjuvant or palliative treatment [4, 6].

Cancer cells are more radiosensitive due to their constant mitotic activity, although healthy cells can also be affected, leading to dose-dependents side effects [4, 6]. One of the indirect side effects is the radiation-related caries, characterized by presenting a painless, rampant and rapid progression [4, 7-9].

Radiation caries have the same etiology than caries in non-irradiated populations with hyposalivation [10], however latest *in vitro* studies show that the tooth hard tissues can be affected by the ionizing radiation, changing their composition, morphology and mechanical properties [4, 7, 9].

Radicular dentin is less mineralized, porous and presents a high water and organic material content, hence being more prone to caries development [11]. Population aging and consequent greater exposure of this tissue in the oral cavity, indicates the necessity of special care to this area during and after radiotherapy.

The chemical characterization of tooth hard tissue can be performed using the Fourier Transform Infrared spectroscopy (FTIR) [12]. The IR radiation promotes vibration of molecular bonds, which are intrinsic of physicochemical properties of the molecule. Hardness is one important mechanical property for tooth fracture evaluation, where the microhardness testing can be performed [13]. In this technique, the testing force per area is calculated in indentation.

To the date, dentin and enamel radiation-induced alterations have controversial results [7, 14-16], where there is a lack of studies relating these changes with radiation caries pathogenesis. In this way, this study aimed to assess the compositional and mechanical changes of radicular dentin when submitted to radiotherapy, using FTIR spectra processing and microhardness testing evaluation, respectively.

II. Materials and Methods

A. Sample preparation

After the approval of The Ethical Committee in the Use of Animals of Federal University of ABC (CEUAx N 9614190917), it was conducted an *in vitro* study with 45 bovine teeth roots, where 90 radicular dentin slabs of 4 x 4 cm were obtained. The samples were sanded to remove surface irregularities and maintained in closed humid recipients under refrigeration between next procedures.

B. Radiotherapy simulation

The samples were immersed in artificial saliva (1.5 mM calcium, 0.9 mM phosphate, 0.1 M tris-HCl, pH 7.0) and submitted to radiotherapy. A cobalt-60 unity (Theratron Phoenix External Beam Therapy System, Best Theratronics Ltd., Ottawa, Canada) was used with 1 Gy/min dose rate for two minutes per day. The irradiation was repeated for 5 days in a row, during 6 weeks, thus this fractional dose resulted in a cumulative dose of 60 Gy. These parameters are commonly used in head and neck cancers radiotherapy protocols [7, 17]

C. Infrared spectroscopy

The infrared spectra were acquired before (control) and after the radiotherapy, using a Frontier FTIR spectrometer (Perkin Elmer, Massachusetts, USA) with Attenuated Total Reflection (ATR) technique. It was selected a 4 cm^{-1} spectral resolution, 4000 to 700 cm^{-1} spectral range and 80 scans mean per sample. The background spectra were acquired in every 5 samples.

To analyze the spectra, a specific algorithm was developed in MATLAB environment. First, the baseline was subtracted and the spectrum was normalized by the phosphate maximum. Then, six IR fingerprint absorption bands were integrated: amide I (1720 to 1593 cm^{-1}), amide II (1580 to 1510 cm^{-1}), amide III (1296 to 1181 cm^{-1}), superposition of the stretching ν_3 and bending ν_4 vibration modes of carbonate (1510 to 1300 cm^{-1}), ν_2 vibration mode of carbonate (887 to 800 cm^{-1}) and phosphate (1181 to 887 cm^{-1}). The phosphate area was used to normalize all other areas.

D. Microhardness testing

The microhardness testing was performed before (control) and after the radiotherapy, using an HMV-2T tester (Shimadzu, Kyoto, Japan) with a Knoop indenter. It was accomplished five indentations, regularly spaced by 100 µm, with a static load of 10 g for 5 seconds.

E. Statistical analysis

The distribution and normality of variances were tested using D'Agostino-Pearson and Shapiro-Wilk methods. It was used paired two-tailed Student's t-test for parametric testing and paired two-tailed Wilcoxon for nonparametric testing. For all tests, it was considered a level of significance of 5%.

III. Results

The FTIR analysis is shown in Fig. 1. The amide III and the carbonate $\nu_3\nu_4$ mean area values decreased after the radiotherapy, presenting a statistically significant difference between before and after the procedure. Amide I, amide II and carbonate ν_2 areas presented no significant difference in the before and after radiotherapy comparison.

The Fig. 2 shows the microhardness testing analysis. Knoop microhardness decreased 38% after the radiotherapy, presenting a statistically significant difference between before and after irradiation.

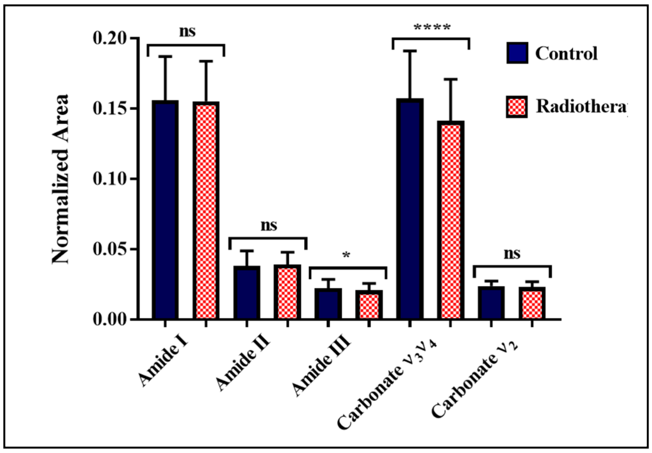

Fig. 1. Before (control) and after radiotherapy mean values of phosphate-normalized areas from infrared spectra. Error bars show standards deviations. Stars (*) denote the statistically significant differences levels; ns – not significant.

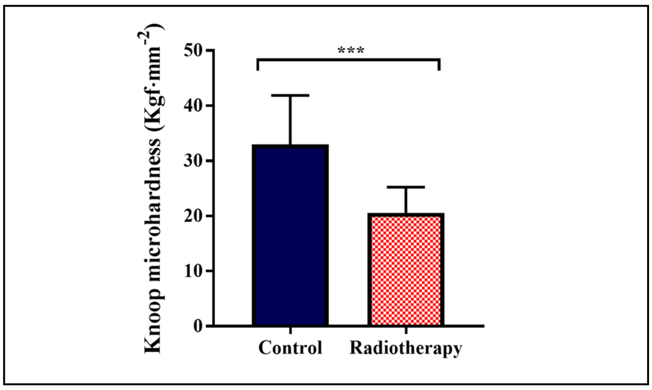

Fig. 2. Knoop microhardness mean values before (control) and after radiotherapy. Error bars show standards deviations. Stars (*) denote the statistically significant difference level.

IV. Discussion

Previous studies present controversial FTIR results. Rodrigues *et al.* [14] observed an augmentation over all absorption bands when applying total cumulative dose of 72 Gy on dentin samples immersed in distilled water. On the other hand, Qing *et al.* [16] and Marangoni-Lopes *et al* [15] demonstrated a decrease of the carbonate absorption bands after 60 and 30 Gy cumulative doses, respectively, on dentin samples immersed in artificial saliva, agreeing with our findings.

The ionizing radiation dissociates water molecules (radiolysis), leading to the formation of reactive free radicals, such as peroxides. These radicals can act as strong oxidants, promoting tissues alterations, mostly in organic compounds [14, 15], and activating collagen-denaturant enzymes [15, 18, 19]. The dentin is composed of a significant water content (11% in weight) [20], hence, being susceptible to radiolysis effects. However, Marangoni-Lopes *et al.* quantified higher amounts of phosphate and calcium in the saliva solution after the irradiation, substantiating their proposal of inorganic compounds also being affected by strong oxidants. This information corroborates with our findings, where the inorganic loss is evidenced on the carbonate $\nu_3\nu_4$ area decrease.

The absorption band of carbonate ν_3 and ν_4 vibration modes are influenced by amide II band, due to collagen helix structure [21, 22]. In this same region, there is also an absorption band related to proline and hydroxyproline, two collagen amino acids [14]. Thus, the carbonate $\nu_3\nu_4$ area decrease can also be related to organic alterations.

Amide III is described as a complex and instable absorption band, due to its dependence of side chains and hydrogen bonds. The radiotherapy can induce a decarboxylation of these side chains, which are responsible for the interaction of the collagen with hydroxyapatite crystals [14, 23].

Microhardness is a mechanical parameter evaluated in tooth exposed to head and neck cancers radiotherapy, but previous studies provides different results, since it is dependent of some variables, such as animal model, aging, sample preparation and loading parameters [14, 15, 19, 24, 25].

Previous studies demonstrate that the dentin microhardness progressive decreases according to the ionizing radiation dose increase [15, 24]. These findings agree with our study, where a decrease of Knoop microhardness was observed after the radiotherapy.

The radiotherapy-induced radicals can promote denaturation or reorganization of the compounds present in collagen composition, leading the tissue to be more prone to cracks formation and compromising the enamel support [15, 24-26]. Mineral content contributes to the dentin mechanical properties [13], thus our microhardness results can also be related to the inorganic alteration evidenced in the FTIR analysis.

The results presented in this study demonstrated that the 60 Gy fractional dose promotes both compositional and mechanical alterations, indicating that this radiotherapy protocol initiated a demineralization process in the radicular dentin. This is an essential information for future studies, where induced caries will be assessed in relation to the radiotherapy effects.

V. Conclusion

According to the methodology in this study, it is possible to conclude that the radiotherapy promotes both compositional and mechanical changes in radicular dentin, and this fact should be considered in future studies.

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