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Radiotherapy effect on nano-mechanical properties and chemical composition of enamel and dentine

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ABSTRACT

Objective: To understand radiotherapy-induced dental lesions characterized by enamel loss or delamination near the dentine–enamel junction (DEJ), this study evaluated enamel and dentine nano-mechanical properties and chemical composition before and after simulated oral cancer radiotherapy.

Design: Sections from seven non-carious third molars were exposed to 2 Gy fractions, 5 days/week for 7 weeks for a total of 70 Gy. Nanoindentation was used to evaluate Young's modulus, while Raman microspectroscopy was used to measure protein/mineral ratios, carbonate/phosphate ratios, and phosphate peak width. All measures were completed prior to and following radiation at the same four buccal and lingual sites 500 and 30 μm from the DEJ in enamel and dentine (E-500, E-30, D-30 and D-500).

Results: The elastic modulus of enamel and dentine was significantly increased ($P \leq 0.05$) following radiation. Based on Raman spectroscopic analysis, there was a significant decrease in the protein to mineral ratio (2931/430 cm^{-1}) following radiation at all sites tested except at D-500, while the carbonate to phosphate ratio (1070/960 cm^{-1}) increased at E-30 and decreased at D-500. Finally, phosphate peak width as measured by FWHM at 960 cm^{-1} significantly decreased at both D-30 and D-500 following radiation.

Conclusions: Simulated radiotherapy produced an increase in the stiffness of enamel and dentine near the DEJ. Increased stiffness is speculated to be the result of the radiation-induced decrease in the protein content, with the percent reduction much greater in the enamel sites. Such changes in mechanical properties and chemical composition could potentially contribute to DEJ biomechanical failure leading to enamel delamination that occurs post-radiotherapy. However, other analyses are required for a better understanding of radiotherapy-induced effects on tooth structure to improve preventive and restorative treatments for oral cancer patients.

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Abbreviations: DEJ, dentine–enamel junction; Gy, gray; E-500, enamel 500 μm from the DEJ; E-30, enamel 30 μm from the DEJ; D-30, dentine 30 μm from the DEJ; D-500, dentine 500 μm from the DEJ; FWHM, full-width at half-maximum; PBS, phosphate buffered saline; ANOVA, analysis of variance; ν, Poisson's ratio; *E*, elastic modulus.

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1. Introduction

Radiotherapy is routinely prescribed to treat patients diagnosed with oral cancer. However, multiple radiation-induced complications occur after radiotherapy treatment such as mucositis, taste loss, xerostomia, and severe dentition breakdown that can result in loss of masticatory function.[1–5] Radiation-induced dentition breakdown begins to occur within the first year following radiotherapy and over time becomes more severe.[1] Post-radiation lesions differ in location and pattern of development and progression as compared to caries in non-radiated patients. For example, instead of pits, fissures and inter-proximal sites, post-radiation dental lesions develop at cervical, cuspal, and incisal areas, sites exposed to occlusal loading and associated flexure and considered more resistant to dental decay. Additionally, post-radiation lesions develop with initial enamel loss that can potentially result in partial to total enamel delamination leaving the exposed dentine vulnerable to subsequent decay.[6,7]

Various factors likely contribute to post-radiation dentition breakdown but it has previously been thought to be an indirect effect due to irradiation-induced changes in salivary gland tissue resulting in hyposalivation.[1,2,5] However, we completed a clinical study and reported that the severity of dentition breakdown is also linked to the individual tooth dose with three tiers of tooth dose–response.[8] Minimal tooth damage occurs below 30 Gy; there is a 2 – 3x increased risk of tooth breakdown between 30 and 60 Gy likely related to salivary gland impact; and a 10x increased risk of tooth damage when the tooth-level dose is >60 Gy indicating radiation-induced damage to the tooth in addition to salivary gland damage. These findings suggest a direct effect of radiation on tooth structure with increasing radiation dose to the tooth.

To better understand radiotherapy-induced dentition breakdown distinguished by biomechanical failure of the dentine–enamel junction (DEJ) leading to enamel delamination, our group is focused on characterizing the structure, properties, and composition of enamel and dentine associated with the DEJ as well as post-radiation changes to those tissues. We recently demonstrated a protein-based enamel matrix layer containing type VII and type IV collagen that bridges with the DEJ in adult teeth.[9–11] This organic matrix layer is distributed along the enamel inner region, appears proportional in thickness to the anatomical enamel layer, and may play a role in stabilization of the DEJ. Next steps are to evaluate any radiation-induced changes that might occur within the enamel and dentine associated with the DEJ. While there have been previous evaluations of mechanical properties of enamel and dentine following in vitro radiation, the results are not consistent. Some studies reported changes in dentine and enamel of extracted tooth specimens radiated with doses greater than 60 Gy.[12–15] Another recent study reported mechanical property changes in enamel at lower in vitro doses (10–30 Gy) but no significant change at higher doses (40–60 Gy), while dentine mechanical property changes occurred at doses ranging from 10 to 60 Gy.[16] Conversely, other studies stated there was no significant change in mechanical properties[17] or chemical composition[18] of enamel and dentine following radiation to sterilize extracted teeth. Nevertheless, there is great variability within the experimental methods of these various studies including differences in storage time and storage solution of the tooth specimens that could affect results[19–21] as well differences as to how and where the tooth properties were measured, another factor that could affect results.[22–24] Finally, another important source of variability is the differences between teeth between patients and even within the same patient.[25–27]

Although previous studies correlated chemical structure with mechanical properties of non-radiated teeth,[28–30] similar studies have not been done to evaluate the effects of radiotherapy on tooth structure. With the combined use of nanoindentation and Raman microspectroscopy, we proposed to measure nano-mechanical properties and chemical composition of teeth from similar positions on the same tooth before and after radiation simulating oral cancer radiotherapy.

2. Materials and methods

Seven non-carious third molars previously extracted from individuals aged 18–20 years old were collected according to a protocol approved by the University of Missouri-Kansas City adult health science institutional review board. Excess soft tissue was removed and the teeth were stored at 4 °C in phosphate buffered saline (PBS, pH 7.4) with 0.002% sodium azide as a microbial inhibitor. A slow-speed water-cooled diamond saw (Buehler Ltd., Lake Bluff, IL, USA), was used to remove the roots from the molars. The remaining crowns were then sectioned buccolingually to generate a 2-mm-thick cross-sectional slice centred on the mesiobuccal and mesiolingual cusps. After initial nanoindentation testing and Raman microspectroscopy, the sections were adhered in an upright position to a small glass cover slip (Thermo Scientific, Portsmouth, NH, USA) using sticky wax (Hygenic Corporation, Akron, OH, USA). Individual tooth sections were placed into a 20 ml scintillation vial (MidSci, St. Louis, MO, USA). Teeth sections were irradiated in a Varian 2100iX linear accelerator using an energy of 6 MV photons (Kansas City Cancer Centers, Kansas City, KS). To simulate oral cancer radiotherapy, teeth sections were exposed to 2 Gy fractions, 5 days a week for 7 weeks for a total of 35 fractions equal to 70 Gy (frequent oral cancer dose). Additionally, to simulate reduced intraoral moisture conditions experienced by patients, enough PBS was placed in the vial to cover the slide but not submerge the tooth section. Following radiation, teeth sections remained in the vials with the same minimal amount of PBS and were stored at 4 °C.

2.1. *Nanoindentation*

Nanoindentation analyses were completed before and following radiation. Prior to analyses, the sections were sequentially polished under water using 600- and 1200-grit SiC paper and a ChemoMet polishing cloth (Buehler Ltd.). For both pre- and post-radiation analyses, tooth sections were evaluated at four buccal and lingual sites on a line located directly adjacent to the lowest portion of the occlusal fossa DEJ and parallel to the occlusal cusps. The sites were positioned 30 μm and 500 μm away from the DEJ in both enamel and dentine, E-500, E-30,