Scanning electric microscopy used to analyze the effect of gamma irradiation on enamel and dentin

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Scanning electron microscopy (SEM) has been extensively used in enamel and dentin surface morphological examinations following several challenges. Irradiation treatment significantly decreases the ultimate strength and modifies the structural morphology of dentin and of enamel. The effect of gamma irradiation therapy on enamel and dentin in relation to prism orientation, dentin tubule orientation, and location can be determined using SEM. Understanding the relationship between gamma irradiation and tooth substrate can be an important key to draw treatment protocols to prevent damage to enamel and dentin in patients with head and neck cancer. The ultimate strength mechanical test can expose inner enamel and dentin, and using SEM analysis is possible to predict where the failure initiate and which structures are more or less influenced by radiotherapy. This paper describes the use of the SEM to verify the effect of gamma irradiation on enamel in relation to prism orientation and coronal and root dentin in relation to tubules orientation. These findings could help the researchers in this area to discover the adequate treatment to prevent damage to enamel and dentin.

Key words: enamel; dentin; irradiation; scanning electric microscopy; mechanical properties

1. Introduction

The tooth consists of mineralized and non-mineralized epithelial and connective tissues which provide their physical properties. The anatomic crown is covered by enamel, the hardest and the most mineralized tissue in the human body, which is supported by dentin, an elastic, avascular, hard connective tissue [1-3]. The root is formed by dentin attached to the cementum, which are mineralized tissues showing inorganic content similar to bone [3-5]. The dentin is formed from and supported by the dental pulp, a soft connective tissue, sum set the pulp-dentin complex [5].

Microstructural organization and relative composition of organic, mineral, and water phases determine the mechanical properties of mineralized dental structures [2,6]. The high mineral content, nearly 96%, makes enamel extremely hard and brittle [1], susceptible to cracks and fractures during physiologic masticatory loads. However, the dentin, which presents higher resilience and lower modulus of elasticity, contributes to maintain the enamel integrity, dissipating stress [7]. The collagen content and dentin tubule orientation contributes to the singular response to tensile strength showed by dentin [6] and lower microhardness as compared with enamel [3,4]. The huge volume of inorganic enamel matrix occupied by crystalline calcium phosphate, densely packed as hydroxyapatite crystals, differentiates it from other mineralized tissues [1,3].

Enamel microstructure is generate as the result of highly controlled interactions between specialized enamel proteins and tooth minerals [3,4]. Though a part of extracellular matrix proteins are degraded during the maturation phase, the remained proteins interact with crystals forming a lacy network of organic material [3], which have profound effects on enamel toughness and carious development and progression [3,4]. The crystals alignment create structures termed rods, frequently designated as enamel prisms, separated by an interrod substance, which consists of apatite crystallites aligned in a different direction from the rods [1,3]. These complex structures result in an intricate pattern of variations in crystal orientation and have influence on the weakness of enamel around or between rods observed by ultimate tensile strength (UTS) tests [2]. Several researches demonstrated that changes on rod or prism direction observed in different crown regions have clinical importance, reflecting on enamel resistance [2,9].

Notably, oral cancer was recently included among the World Health Organization’s (WHO) priorities for action [10]. Leading into account that gamma irradiation is a treatment modality largely used for head and neck malignancies [11], either alone or combined with surgery and chemotherapy [12]. Nevertheless there is minimal data available as to the effect of radiotherapy on the teeth structure [9,13,14]. It has been observed that after irradiation the mechanical properties of teeth are altered. Smooth enamel surfaces, normally resistant to decay, are affected and caries development and progression are accelerated [15].

The effect of radiotherapy on enamel and dentin in relation to prism orientation, dentin tubule orientation, and location can be elucidated to improve treatment protocols and to prevent damage to enamel and dentin in patients under radiotherapy [9]. Scanning electron microscopy (SEM) associated to ultimate strength mechanical test (UTS) is a useful
tool to evaluate the effect of gamma irradiation on enamel and dentin microstructure, helping to predict where the failure initiate and which structures are more or less influenced by radiotherapy [9]. This paper describes the use of the SEM to verify the effect of gamma irradiation on enamel in relation to prism orientation and coronal and root dentin in relation to tubules orientation.

2. Material and Methods

Ten sound human third molars free of caries, from individuals 18 to 23 yrs old, were collected with informed donor consent (After approval by the Ethical Committee of the Federal University of Uberlândia-MG, Brazil, under the protocol 328-08). The teeth were stored in a solution of 0.2% thymol for no longer than 1 mo after extraction (Fig 1.1), then cleaned of gross debris and placed in distilled water for 24 hrs before the beginning of the experiment. To facilitate the simulation of mouthwash, the roots were embedded in blocks of silicone 2mm below the cementum enamel junction (Fig 1.2). The teeth were divided into 2 groups: an irradiated group (Ir) (number of specimens, N = 05) and a non-irradiated group (NIr) (N = 05).

In the irradiated groups, the samples received 60 Gy of gamma radiation in a 60Cobalt irradiation unite (Theratron Phoenix 60Cobalt Radiotherapy Treatment Unit - Theratronics International, Ltd., Atomic Energy of Canada, Ltd., AECL Medical, Ontario, Canada), fractioned in daily values of 2 Gy, five days a week (Fig 1.3). The dose is defined on the radiotherapy unit panel, that self-measure the irradiation level emitted [9]. Both groups were stored in artificial saliva changed daily.

After irradiation or not, to facilitate further enamel slicing and testing was formed an extension of the crown [2]. The intact occlusal enamel surface was etched with 37% phosphoric acid (Dentsply Caulk, Milford, DE, USA) for 30s, rinsed and bonded with Adper Single Bond 2 (3M-Espe, St Paul, MN, USA). A resin composite block (4 mm high) was built up in three layers with TPH Spectrum resin composite (Filttek Z250, 3M-Espe, Saint Paul, MN, USA). Each increment was light-polymerized for 20 s and the specimens were stored in distilled water at 37°C for 24 h. Root and crown were then serially, vertically sectioned in a buccal–lingual direction (Fig. 1) to obtain several slices of approximately 1.0mm thick by means of a low-speed diamond saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA) under water cooling. Six slices with 1.0±0.21 mm in thickness were obtained for each root and crown portions and were further trimmed to an hourglass shape that produced cross section tested area with 1.0±0.19 mm². The samples of each tooth were randomly assigned into six groups (number of slices= 20). The slices were trimmed to an ‘hourglass’-shape with a superfine diamond bur #1090 (KG Sorensen, Barueri, São Paulo, Brazil) under air–water irrigation, following the direction parallel and transversal to the tubules and prisms (Fig. 1.4).

Each specimen was fixed to the grips of the microtensile testing device with cyanoacrylate glue (Loctite Super Bonder, Henkel Loctite Corporation, USA) and tested to tensile at 0.5 mm/min in a testing machine (EMIC 2000 DL, São José dos Pinhais, PR, Brazil) until failure. After fracture, the specimen was removed from the testing apparatus and the specimens were allowed to air-dry overnight, sputtercoated with gold (MED 010, Balzers, Balzers, Leichtenstein), and examined by scanning electron microscopy (LEO 435 VP, Carl Zeiss, Jena, Germany). Representative areas of tested sites were photographed at 2500-3000X magnification.
3. Results

Representative SEM micrographs of the fractured site of the specimens are shown in Figs. 2–7. When enamel tested perpendicular to its prismatic orientation, fractures occurred preferentially obliquely to the long axis of the prisms, leaving them prominent on the surface (Fig. 2). Cone-like ends were generally seen at the fractured site of individual prisms for non-irradiated fractured enamel (Fig. 2A). Fractured irradiated enamel tested perpendicular to its prismatic orientation showed also predominantly oblique fracture orientation, however the fracture mode tending to cross to the inter-prismatic region without cone-like ends (Fig. 2B).

The fractured site of non-irradiated enamel tested parallel to its prismatic orientation presented cone-shaped structures (Fig. 3A). Since fracture did not occur in a single plane, these structures appeared to be protruding from the surface (Fig. 3A). The surfaces of irradiated samples stressed parallel to prisms showed more irregular surfaces involving greater interprismatic change (Fig. 3B), demonstrating alteration of interprismatic areas.
Coronal dentin specimens presented a larger area of solid dentin and low tubule density (Fig. 4). Non-irradiated coronal dentin fractured perpendicularly to tubules showed regular planes (Fig. 4A). Samples of irradiated coronal dentin fractured perpendicularly to tubule orientation showed the same plane of fracture (Fig. 4B), but with greater micro-cracks present in peritubular dentin (arrows in Fig. 4B).

Coronal dentin fractured presented fractures did not occur in a sharp, single plane along the intertubular and peritubular dentin (Fig. 5). Fractures across the peritubular dentin occurred either above or below the plane of fracture of the intertubular dentin (Fig. 5). Peritubular dentin protruding from the surface as a distinct structure is more evident in non-irradiated coronal dentin (Fig. 5A). An abrupt change in the plane of fracture was observed at the boundaries between intertubular and peritubular dentin (Fig. 5A). Irradiated dentin fractured parallel to tubule orientation showed more inhomogeneous fracture planes (Fig. 5B), with great numbers of micro-cracks in peritubular dentin (arrows in Fig. 5B).

Fig. 3. Fracture pattern in sites of samples of enamel tested parallel to prism orientation. A. non-irradiated enamel; B. irradiated enamel. Original magnification 2000X.

Fig. 4. Fracture pattern in sites of samples of coronal dentin tested perpendicular to tubules orientation. A. non-irradiated coronal dentin; B. irradiated coronal dentin. Original magnification 2000X.

Fig. 5. Fracture pattern in sites of samples of coronal dentin tested parallel to tubules orientation. A. non-irradiated coronal dentin; B. irradiated coronal dentin. Original magnification 2000X.
Root dentin (Fig. 6) fractured perpendicularly to tubules showed regular planes. In these samples, intertubular dentin was smooth, showing collagen fibrils oriented transversely to the tubule direction. Irradiated dentin fractured perpendicularly to tubule orientation showed several numbers of micro-cracks in peritubular dentin (arrows in Fig. 6B).

Fig. 6. Fracture pattern in sites of samples of root dentin tested perpendicular to tubules orientation. A. non-irradiated root dentin; B. irradiated root dentin. Original magnification 2000X.

Root dentin (Fig. 7) fractured parallel to tubules showed more homogeneous fracture planes than coronal dentin (5). It is more difficult to differentiate the peritubular and intertubular dentin (Fig. 7). Irradiated dentin fractured showed several numbers of micro-cracks in peritubular dentin (arrows in Fig. 7B).

Fig. 7. Fracture pattern in sites of samples of coronal dentin tested parallel to tubules orientation. A. non-irradiated coronal dentin; B. irradiated coronal dentin. Original magnification 5000X.

4. Discussion

The tooth is the only mineralized organ that is located partially internal and partially external to the body. To minimize wear during function, the tooth includes highly mineralized tissues that present physical properties based on their composition and micro morphology [1]. Head and neck includes cancer of oral cavity, pharynx, and larynx has been recognized as a significant component of the global burden of cancer [16]. Radiotherapy is a treatment protocol widely used for treatment of cancer [11]. Despite the advantage of preserving tissue structure, radiotherapy causes adverse changes in the oral cavity [17] as mucositis, candidiases, dysgeusia, radiation caries, osteoradionecrosis and xerostomia [18]. The mechanical properties and micromorphology of teeth are clearly changed by a tumour therapeutic irradiation with high photon energy [14].

According to the methodology used in this study several aspects were taken in account to standardize the evaluation and approximate the in vitro reality closer to clinical conditions. The protocol used for radiation therapy was the same as that normally used for the treatment of patients with head and neck cancer; the incremental fraction dose was important to simulate the same dose that patients receive daily. During radiation, teeth were stored in artificial saliva. Two studies carried out by our groups used different media to store teeth during irradiation protocol, distilled water [9] and artificial saliva [19]. The results of both studies were statistical similar regarding SEM analysis and UTS test.

The UTS of enamel is significantly higher when tested parallel to the orientation angles regardless of the presence and type of radiation treatment [2,9]. The weaker interprismatic substance is aligned perpendicular to the load, so the
tension rapidly propagates across the specimen causing it to fail under a lower load. Fig. 2 confirms that enamel failure occurred by separation of prisms along the interprismatic substance, only a few prisms are obliquely cleaved [2]. On enamel stressed parallel to its prismatic orientation the stress concentrate on the stronger prismatic units and the fracture requires all prisms to be cleaved before catastrophic failure occurs (Fig 3), resulting in significantly higher UTS for enamel when tested under this condition [2,9]. Moreover, the interprismatic region to present more organic content is more influenced by radiotherapy [20], higher reduction on resistance of stressed perpendicular enamel than enamel stressed parallel to prisms orientation exits. SEM analysis can clearly demonstrate this difference between enamel irradiated and no irradiated. The surface of enamel, regardless to prism orientation, when submitted to radiotherapy presented a melted appearance (Fig 2B and 3B). Recent study conducted by our group [19] demonstrated that the use of fluoride 0.05% used as a mouthwash 3 times a day during radiotherapy protocol can prevent the damage maintaining the morphology of the enamel similar to non-irradiated enamel.

Protein phase is concentrated in the interprismatic region, on the other hand the UTS has been related to be lower in the perpendicular prismatic plane than in the parallel plane. These aspect confirm that protein phase of the enamel is more influenced by radiotherapy than mineralized phase [20]. We identified a greater degree of irregularity in the fracture pattern of irradiated than non-irradiated enamel (Fig 2 and 3). This can possibly be explained by a ‘disarrangement’ of the crystalline portion (Fig 3B) of enamel [21], which represents the restructuring of the chemical bonds into the mineral components that alter the crystalline organization and alterations of protein interprismatic links [20], which resulted in higher prism cleavage independent of stress orientation.

Dentin is a hydrated biological composite composed of 70% inorganic material, 18% organic matrix and 12% water (wt%), with properties and structural components that vary with location [5]. The structural composition of dentin includes oriented tubules surrounded by a highly mineralized cuff of peritubular dentin and an intertubular matrix consisting of type I collagen fibrils reinforced with apatite [22].

Most of the tooth structure is comprised of dentin. The tubule density and the area occupied by solid dentin vary with distance from the pulp chamber to the dentin-enamel junction [23]. Several studies have investigated the relationship between microstructure and the mechanical properties of dentin [2,6,9]. Results indicated that cohesive strength of dentin varies significantly and is dependent on intra-tooth location [2].

Higher UTS values when tensile force was yielded directed perpendicular to tubule orientation [9]. It has been reported that the majority of the fibrils run either perpendicular or oblique to the tubule direction [8], so tensile force applied to collagen fibrils longitudinally results in higher UTS values than that applied perpendicularly. The results are consistent that fiber orientations of coronal and root dentin, which involve the tubules perpendicularly in the long axis. The mechanical properties of dentin are defined partly by its organic components [8,24], so when dentin is stressed perpendicular to tubule orientation, this substrate is really stressed parallel to fiber orientation, which, since the fibers are stretched, results in increased load to failure.

Dentin presented different characteristics after radiotherapy treatment when compared with non-irradiated dentin [9]. While the mineral concentration is higher in peritubular dentin, the microcracks are concentrated in this area (Fig. 4B, 5B, 6B and 7B), reducing UTS values in coronal and root dentin [9]. The irradiation promotes side chain decarboxylation and a loss of acidic phosphate groups with formation of new calcium ion bridged phosphate groups [16]. The mineral-organic interaction between apatite and collagen is reduced and may induce micro cracks (Fig. 4B, 5B, 6B and 7B) in the hydroxyapatite mineral [25]. Additionally, the denaturation of the organic matrix caused by radiolysis would reduce the physical anchorage between enamel and dentin and the inner stability of dentin, since it contains collagen fibers as well [26].

In the protocol of dental care before, during and after radiation treatment is essential to use of fluoride to reduce the incidence of caries radiation [27] and dentin and the use of chlorhexidine to reduce the intensity of mucositis caused by anticancer therapy [27]. Recent study conducted by our group [19] provided that the rinsing with chlorhexidine 0.12% recover partially the damage of the mechanical properties of coronal dentin irradiated and that rinsing with sodium fluoride to 0.05% recover the mechanical properties of enamel irradiated similarly to the non-irradiated enamel, therefore the use of these substances during radiation treatment becomes even more important in an attempt to reduce the side effects and improve the quality of life of patients with cancer of head and neck. The scanning electric microscopy is a powerful methodology that can be easily conducted and clearly help health area to clarify the mechanism of the diseases present in oral environment.

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