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NANOMECHANICAL PROPERTIES OF DENTAL SURFACES STUDY AS AN IRRADIATED TREATMENT SIMULATION

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Abstract. Patients with head and neck cancers are usually subjected to radiotherapy, using therapeutically doses of 60Gy. The purpose of this study was to investigate the effect of gamma irradiation on hard dental tissues hardness. Three human teeth have been prepared in the form of discs according to AFM sample preparation and divided in two groups: control group, respectively ^{60}Co irradiated group. In order to reduce the risk of fissures the hardness of enamel and intertubular dentine was evaluated using nanoindentation coupled with AFM technique. The mean hardness values decreased for both enamel and dentin when comparing nonirradiated-irradiated teeth. Also information about DEJ width, dentinal tubules diameter and depth were obtained. This study confirms the applicability of AFM nanoindentation for studying biological samples.

Key words: radiotherapy, gamma irradiation, AFM, hardness.

1. INTRODUCTION

Tooth enamel is the most mineralized tissue of human body. Its composition consists of 96 wt% inorganic material, 4 wt% organic material and water. The inorganic part represents 70 wt% in dentin [1]. Human enamel is an anisotropic composite, consisting of aligned prism rods with protein sheath. Each prism rod contains multiple hydroxyapatite single crystals, separated from each other by enamel [2]. The mechanical behavior and reliability of enamel have been major concerns.

Patients with head and neck cancers are usually subjected to radiotherapy, using therapeutically doses of 60 Gy. Post-irradiation quality of life can be drastically diminished due to numerous radiation-induced oral complications, such as: hypo salivation, microsites, severe dentition breakdown (radiation caries) and loss of masticatory function [3–7].

Many factors contribute to tooth damage following radiotherapy, but the most important is xerostomia [5–7]. This *in vitro* study intends to show, using atomic force microscopy technique, that therapeutic doses of radiation have a direct effect on mineralized tooth structure, affecting the hardness of the hard dental tissues.

Atomic Force Microscopy (AFM) is a surface topography technique which provides a three dimensional image by measuring forces between a sharp tip and a surface at very short distance [8]. This technique can solve surface structure down to the nanometer scale, but the great advantage is that is not limited to conductive and semiconductive samples like SEM [9]. Therefore it can analyze almost any type of material: metals, polymers, semiconductor, ceramics, cells [10], biological materials [11], thin film [12].

In this preliminary study atomic force microscopy was used to obtain the surface topography of the human tooth-enamel and dentine. The direct effect of gamma irradiation on dentine and enamel hardness was also investigated using nanoindentation technique.

2. MATERIALS AND METHODS

2.1. TOOTH PREPARATION AND IRRADIATION

Three sound teeth extracted from patients with ages between 45–60 years old the group of age most affected by cancers in the region of head and neck were used in this *in vitro* study. Teeth were stored in saline solution NaCl 0,9%, changed daily. They were serially sectioned horizontally in a buccal-lingual direction under wet condition to prevent dehydration (Buehler Precision Saw). Two coronal slices of approximately 2.0 mm thick contain enamel, dentine-enamel junction (DEJ) and dentine were obtained for each tooth and divided into two groups – control group and irradiated group, Figure 1.

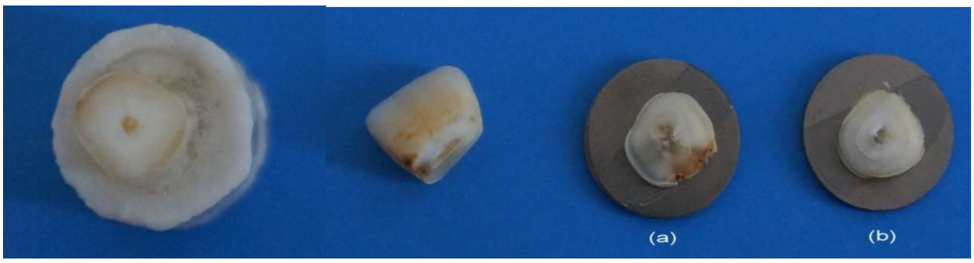

Fig. 1 – Human teeth preparation for AFM analysis. Teeth were divided into two groups: a) control group – non irradiated samples; b) ^{60}Co irradiated group.

After that, in order to obtain a flat and uniform surface needed for nanoindentation analysis, specimens were embedded in duracryl and polished on microcloth 10″ using alumina suspension down to 0.05 μm (Buehler grinder polisher). Finally alumina suspensions were removed from the specimen surface by ultrasonic treatment in distilled water for 30 minute. There are many studies emphasizing the importance of biological sample preparation, dehydration and choosing a suitable hydrating fluid [13] thereby samples were kept in distilled water until AFM analysis to prevent a possible modification of the mechanical properties.

In order to simulate *in vivo* conditions samples were irradiated at IFIN-HH (Irradiations Technology Center IRASM) at the average absorbed dose of 70 ± 9 Gy. The dosimeter system was ALANINA/RPE – RISØ-HDRL, Denmark –>NPL, England (Certificate 06C-51).

2.2. NANOINDENTATION

Nanoindentation is a suitable technique for determining the mechanical properties of hard biological tissues bone and teeth [14]. Although nanoindentations only examine a thin surface layer (< 1 μm), the mechanical properties obtained are assumed to be representative of the bulk material [15]. Nanoindentation experiment under dry conditions was performed on dentine and enamel near the dentine-enamel junction using a MultiMode NanoScope IIID Controller. All measurements were carried out at room temperature using a diamond tip stainless steel cantilever. This tip has a spring constant of 217 N/m, a resonant frequency of 63 kHz and a tip radius less than 25 nm to ensure good imaging resolution [16].

3. RESULTS AND DISCUSSION

AFM images of the unpolished and polished enamel and dentine are shown in Fig. 2. Trying to get higher quality images with fewer artifacts tapping mode was used [17]. Images were obtained using a 1–10 Ohm-cm Phosphorus (n) doped Si tip and a scanner having a maximum range of 125 μm × 125 μm (*x*, *y*) and a vertical range (*z*) of 5 μm. The topography, surface roughness and DEJ width were evaluated using the V531r1 AFM software. Roughness values were obtained over the entire image. After polishing, roughness parameter such as Root Mean Square (Rq) significantly decreases from 80 nm to 3.3 nm for enamel respectively from 280 nm to 102 nm for dentine. Dentine tubules surrounded by peritubular and intertubular dentine are visible on dentine polished surface. The maximum dentinal tubule diameter is about 1.8 μm (Fig. 3).

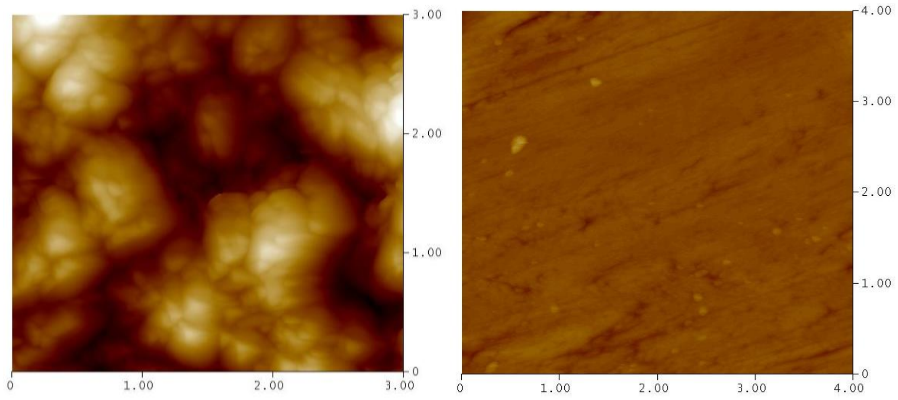

Fig. 2 – Tapping Mode AFM images of the unpolished/polished enamel. Roughness decrease significantly from 80 nm to 3.3 nm.

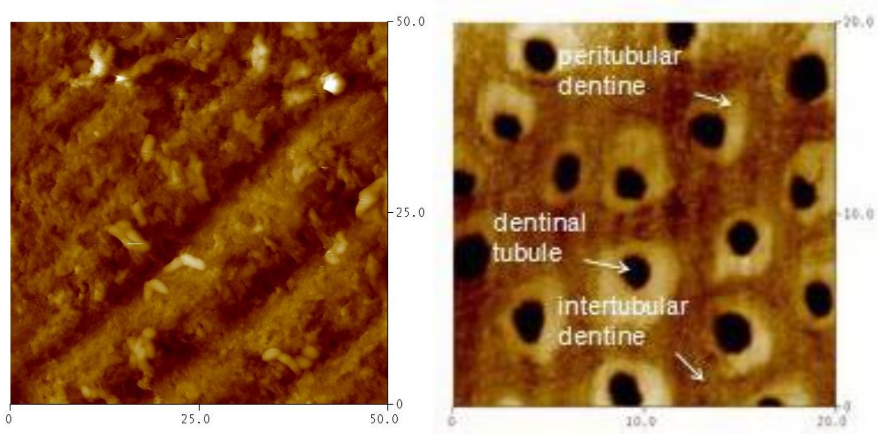

Fig. 3 – Tapping Mode AFM images of the unpolished/polished dentine surface. The polished surface shows the dentinal tubules surrounded by peritubular and intertubular dentine; the maximum dentinal tubule diameter is about 1.8 μm.

The dentine-enamel junction was clearly visible (Fig. 4). DEJ is a natural junction that unites two mechanically dissimilar calcified tissues: the hard and brittle enamel and the softer and tougher dentin [18]. Studies [19] reported different values for DEJ. In our investigation DEJ width varied from about 6 μm to 20 μm.

The main purpose of this preliminary study was to determine the direct effect of 60 Gy gamma irradiation – the usual dose used in the treatment of head and neck cancers – on hard dental tissues. A matrix consisting of six indentations was made on enamel and dentin near the dentine-enamel junction using the Auto Indent option under exactly the same conditions (Fig. 5). Indentations on dentine were made on the intertubular dentine, while on enamel were made without considering the rod and prisms orientations.

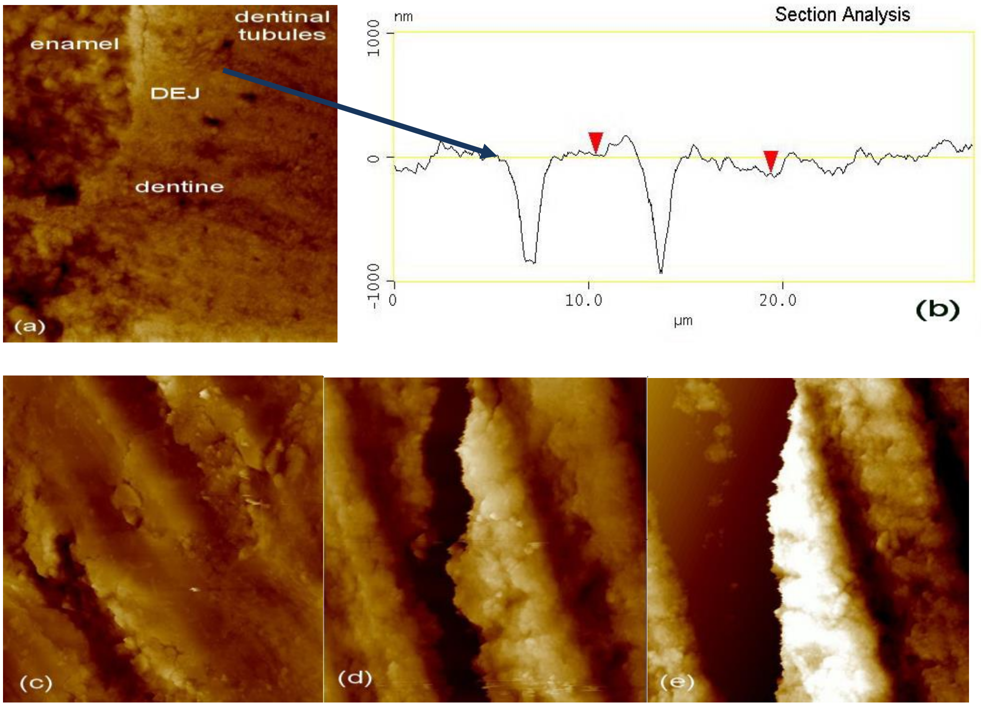

Fig. 4 – TM AFM images showing the tooth structure: a) enamel, dentine-enamel junction (DEJ), dentinal tubules; b) the maximum dentinal tubules depth is around 1000 nm; c) the DEJ is clearly visible; d) its width; e) is about 6 µm respectively 20 µm.

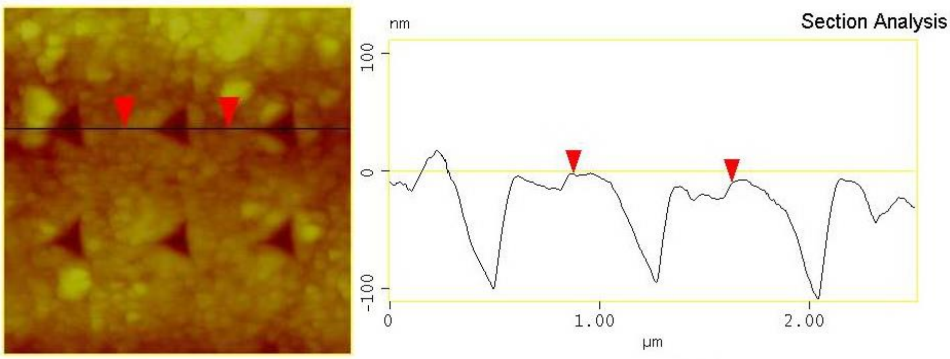

Fig. 5

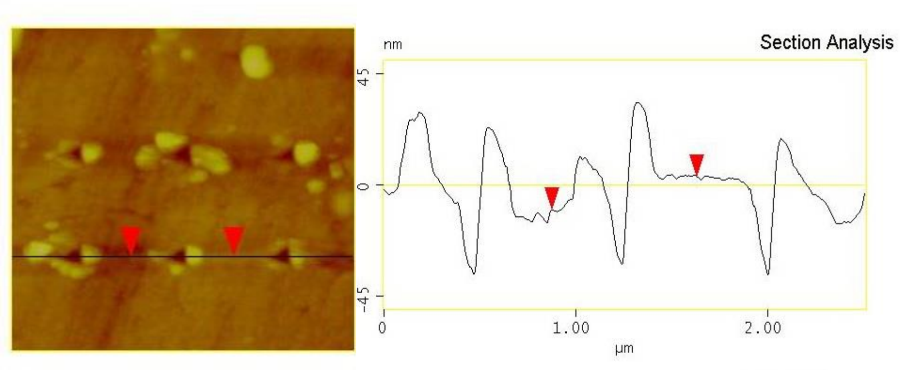

Fig. 5 (continued) – AFM images of array of nanoindentations and section analysis for nonirradiated dentine (up) and enamel (down). The yellow area around the triangle is the pile-up material.

Topography and section analysis of the irradiated dentine and enamel are shown in the figure below. As it can be seen from the section analysis, pile-up effect is more evident for the irradiated samples. According to [20] softer film led to sink-in phenomenon while harder film led to pile-up phenomenon. In our case the pile-up phenomenon is present on the nonirradiated enamel (Fig. 5) which is normal if we consider that enamel is the hardest mineralized surface of the teeth. On irradiated samples is present on both enamel and dentine (Fig. 6).

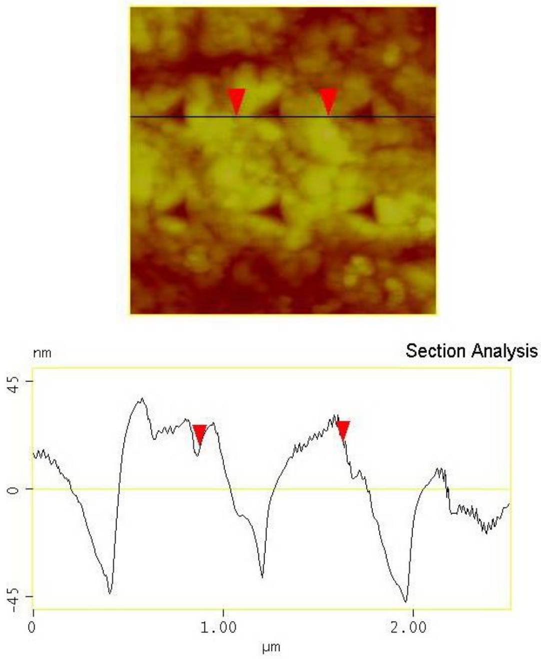

Fig. 6

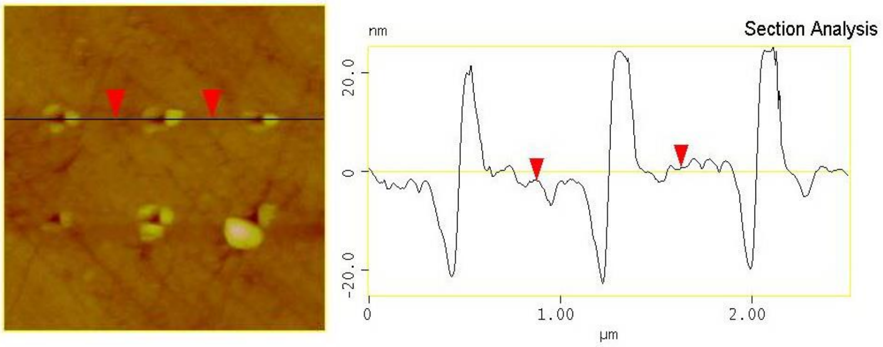

Fig. 6 (continued) – AFM images of array of nanoindentations and section analysis for irradiated dentine (up) and enamel (down). The pile-up effect is evident for both dentine and enamel.

The hardness of the material was expressed by [21]

$$H = F_{\max}/A_P,$$

where F is the maximum indentation force and A is the projected area. The force was calculated using the following formula: cantilever spring constant (227 N/m) multiply by the trigger threshold (2 V), deflection sensitivity (217 nm/V) and by a conversion factor (1×10^{-9} m/1 nm) [16]. The projected area was measured from the AFM image and calculated using Heron formula. Indentation force of 98 µN was applied to both polished dentine and enamel surfaces resulting in maximum indentations depth of about 100 nm for nonirradiated dentine, respectively about 45 nm for nonirradiated enamel.

The hardness values for non irradiated/ irradiated enamel revealed a decrease from 10.86 GPa to 7.01 GPa. The same decrease was register for dentine from 5.63 GPa – nonirradiated samples – to 3.68 GPa irradiated samples. It is well known [22] that the hardness of dentine varies depending on the area: hardness near the DEJ is greater than that near the pulp. Previous studies measured hardness of enamel and established that the regions closest to the occlusal surface have the highest values ($H > 6$ GPa), while the regions near the dentino-enamel junction have the lowest values ($H > 3$ GPa) [23]. Different studies show different values, generally attributed to variations in the microstructure or microchemistry of hard dental tissues. Also, according to other studies [1, 4] the hardness values obtained from dry samples may be higher than those obtained from wet samples.

Radiation caries are a highly destructive form of decay after radiotherapy in the region of head and neck. Hiposalivation, dietary changes and alteration of oral flora are the most important etiological factors, but radiation has also a direct effect on hard dental tissues. The enamel is most susceptible to acid attack, the decrease of hardness registered in this in vitro study being significantly important.

Several studies show that microhardness of dentin and the stability in the region of dentine-enamel junction are affected by radiotherapy [24], resulting in enamel cracks and subsequent microbial colonization.

The reduced hardness of enamel and dentin could be a direct factor leading to radiation caries. Radiation carious lesions have a different pattern than the regular ones. They appear especially in sites exposed to occlusal loading (incisal and cuspal) and associated flexure (cervical), that usually are resistant to typical dental decay [5]. The above mentioned radiation-induced ultra-structural changes raise the caries susceptibility in patients with oral cancers.

4. CONCLUSIONS

AFM nanoindentation has been used in hardness investigation of dentine and enamel. The results show a significantly decrease of the hardness values of nonirradiated *vs* irradiated samples, for both enamel and dentine. Radiotherapy frequently used in the treatment of head and neck cancers induces alterations of the oral environment (xerostomia, mucositis and other complications) but has also a direct effect on hard dental tissues, making them more susceptible to carious lesions. The side-effects of radiotherapy can be reduced to some extent with appropriate prevention and treatment.

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