*ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR – ABEN*
*2019 International Nuclear Atlantic Conference - INAC 2019*
*Santos, SP, Brazil, October 21-25, 2019*

Agência Brasileira do ISBN
ISBN 978-85-99141-08-3
9 788599 141083

# FTIR Analysis of Human Dentin Submitted to Gamma Radiation

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## ABSTRACT

Global data indicate that head and neck cancer express one of the sixth most common types of malignant cancers. In 2030, head and neck cancer predict 1,031,439 new cases per year around the world. Radiation therapy is used as a major therapy step in the treatment protocol for head and neck malignancies. Radiation caries consists in a side effect of xerostomia, ie a considerable reduction in the quantity and quality of saliva in the oral cavity, being defined as a type of cavity that advance severely with fast progression being able to injure the dental pulp. Gamma radiation effects can promote changes associated to modification in the enamel prismatic structure, the reduction in surface microhardness and biochemically alterations in the tooth composition. However, other studies where the human dental enamel using ionizing radiation present no differences found in the integrity of the dental enamel and there was no interference in the surface microhardness. In this study, 20 human dentin samples were evaluated, split randomly into the control group and irradiated group at 25 kGy, in the Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM). The FTIR shows that no significant changes were found in the Phosphate band representing the inorganic material. On the other hand, the Amide I band - chosen as the representative of the organic matrix composed mainly of collagen- showed a significant difference, suggesting a degradation of the organic content. These findings corroborate with SEM analysis after the gamma irradiation dose at 25 kGy.

## 1. INTRODUCTION

Worldwide, the records of malignant head and neck neoplasms, predicts 1,031,439 new cases each year [1]. The most commonly reported malignant lip and oral cavity neoplasia was the oral cavity, oropharynx, nasopharynx, pharynx, larynx, salivary glands, nasal and paranasal sinuses as regarded by the National Cancer Institute (INCA). Those whose primary location is the lips, oral cavity, salivary glands, and oropharynx are a common occurrence. In countries such as the United States, these cases account for 5% of all cancers diagnosed annually, India is one of the countries with the highest prevalence of cases, with an average of 75,000 to 80,000 cases annually [1, 2]. More recently INCA data has estimated that for the years 2018 and 2019, with the occurrence of 600 thousand new cases of cancer, those of the oral cavity represents 5% of the cases. Oral cavity cancer etiology is strongly correlated with tobacco use and excessive alcohol use, as well as ultraviolet solar radiation exposure. Human Papilloma Virus (HPV), inadequate fruit and vegetable diets, bad oral hygiene and other associated social and environmental variables are also associated with etiology [2]. Another classification for head and neck cancer is “head and neck malignant neoplasia” (HNMN) which is considered superior aero-digestive tumors, 90% of these are squamous cell carcinomas derived from the epithelium coating [3].

Treatment may include surgeries, chemotherapy, therapies combination, and radiotherapy. Radiotherapy treatment can cause undesirable impacts on the region of the biological tissue where gamma irradiation is targeted. Co-morbidities may include oral mucositis, changes in the salivary quality and quantity, candidiasis and limitations of movement, as well as chronic effects such as muscular atrophy of the face and salivary glands, osteoradionecrosis, xerostomia, tooth cavities and even periodontal disease [1].

Clinically reported xerostomia as the feeling of 'dry mouth' with a reduction in salivary quantity and quality is a result of the interaction of ionizing radiation in radiotherapy-treated patients. Saliva is one of the main mechanisms of action of the maintenance of hydroxyapatite of dental enamel beside the control of microorganisms. In the oral cavity, excreted saliva is within and around the oropharynx. The mouth starts the digestive process, where saliva performs primordial functions, like digestive cake formation for salivary amylase action and subsequent swallowing. It also performs food solubilization allowing for the dissociation of flavors. Due to the enzymes present in the saliva, it is a significant antibacterial barrier that can inhibit the bacteria's metabolism and adhesion. Especially immunoglobulins secretory IgA that acts in the inhibition of the adhesion of bacteria to the dental enamel, IgG and IgM increases the capacity to phagocyte the microorganisms into the oral cavity. Additionally, the release of lysozyme and glycoproteins in the oral cavity acts as the first line of protection, controlling microorganism activity [4]. Nerve impulses which maintain salivary secretion, are part of the autonomous parasympathetic system and can be stimulated by chemoreceptors through chewing or smelling [5]. Studies show that 80% of the salivary function may be lost after irradiation at doses of 20 Gy, corresponding to 10 radiotherapy sessions and that the damage caused in the salivary glands may be irreversible in doses greater than 30 Gy [6].

Radiation caries is a side effect of xerostomia in patients undergoing radiotherapy treatment. One of the main characteristics of radiation caries is related to the time, where fast, aggressive tooth destruction is observed, with rampant caries, progressing severely in the cervical regions, smooth surfaces and even in the incisal teeth, with high rates of progression that can injure the dental pulp. Clinically they were classified in three ways: type 1, being the most observed type, affecting the cervical of the teeth and extending to the enamel-cemental junction, with destruction of circumferential form that can lead to the complete loss of the crown; type 2 that appear as areas of demineralization on all surfaces of the teeth in the form of generalized erosions and worn occlusal surfaces with loss of incisal structures; and type 3 which is the least common and presents a dark brown color in the dentine with occlusal and incisal [2].

There are organic materials, such as proteins, and inorganic materials, such as sodium, potassium, calcium, and others, in the saliva of non-irradiated patients with dental cavities. Calcium and phosphate levels in a non-ionic form (calcium phosphate) are supersaturated in saliva in relation to HA when the pH is neutral (pH = 7.0). The interaction between biofilm and mouth's bacteria causes an increase in the amount of hydrogen ions ($H^+$) and consequently a reduction in the oral cavity's pH, where critical pH for enamel is about 5.5 and for dentin 6.5. Hydrogen ions can bond with phosphate ($PO_4^{3-}$) resulting in $H_2PO_4^{2-}$ (phosphoric acid) which has unstable bonds that can demineralize the dental enamel when associated with other events [2].

Besides the biochemical factors of dental caries process, the disease is classified as being multifactorial due to some conditions including host and teeth, the microbiota, diet, and time.

Nowadays, it is known that dental caries is related to other factors such as social class (including education, income, behavior, attitudes and knowledge regarding the disease), salivary flow present, buffer capacity, fluoride utilization, time and diet composition present in the oral cavity, mouth's pH and the type of bacterial deposits of living organisms or complete sterilization [2].

Studies using gamma radiation with doses of 25 kGy (for sterilization purposes) report that the effects of this irradiation dose with $^{60}Co$ do not promote changes in surface micro-hardness, however, it causes a chromatic alteration due to the organic material deterioration in dentin mainly in the form of surface-absorbed proteins (less than 3%). It seems not to interfere with the incidence of caries up to the irradiation dose of 25 kGy [7]. When compared to the adverse effects caused by other techniques, gamma irradiation seems to be the most suitable method for the sterilization of hard tissue samples (bone, enamel, and dentin).

Biological tissues were analyzed by Fourier Transformed Infrared Spectroscopy (FTIR), a non-subjective and non-destructive analysis of biological materials, which appears as an excellent option, being very useful in cytological and histological diagnoses through the generation of spectral images. Spectroscopy is the study of the absorption or emission of molecules when electromagnetic radiation interacts with matter, a mapping is generated by the different phenomena associated with each interval of the electromagnetic spectrum. Being possible due to the energies of the electromagnetic radiation, it is possible to analyze them continuously as a function of the wavelength, each wave interval receives a denomination according to its characteristic energy [8]. For samples analysis, absorption of energy in the infrared region is necessary, and this will only be possible if a variation of its dipole moment occurs in the variation during the vibration. The dipole moment variation occurs when the electromagnetic radiation focuses on the same natural frequency of the molecule changing its movement and then returning to its fundamental state of energy. In this variation, the resultant must be different from zero in order to obtain an infrared signal [8]. The transmission mode of the attenuated total reflection (ATR) has as an advantage as there is not much contact of the infrared beams with biological samples [8]. For dentin study, it is possible to analyze the bands of dentin structure, thus investigate the effects of gamma irradiation in structures irradiated by the dose of 25 kGy used for sterilization. In this work, we propose to analyze the morphological changes as well as the organic and inorganic changes after irradiation at 25 kGy in human dentin, aiming to understand the radiation cavities process.

## 2. MATERIALS AND METHODS

### 2.1. Experimental Design

This study was approved by the Human Research Ethics Committee (CAAE 10502518.2.0000.0075). Twenty human dentin samples were evaluated, decontaminated with Thymol at the concentration of 0.64 g/L for 48 hours. All the samples of dentin were cut, brushed, and polished in-plane standardized dimensions 3.0 x 3.0 x 0,5 mm. Then split randomly into G1 control group (n=10) and G2 (n=10) irradiated group. Both groups were evaluated by the Fourier Transform Infrared Spectroscopy with the Total Attenuation Reflection (ATR-FTIR), and the G1 group was evaluated as a reference in the Scanning Electron Microscopy (SEM). Until the gamma irradiation, samples from the gamma group

were refrigerated, and those from the control group were stored humidified and refrigerated. For the samples from the gamma group, the sterilization protocol was performed using the $^{60}Co$ Multipurpose Radiator from the Center of Radiation Technology of the Nuclear and Energy Research Institute of the University of São Paulo, which operates through the irradiation system product overlapping source. The irradiation time of the samples was approximately 2 hours making a total of 2 irradiation cycles of 12.5 kGy / cycle. After the 25 kGy sterilization protocol, they were analyzed for their structural integrity and were also evaluated macroscopically with a stereoscopic magnifying glass, after which the analysis of the ATR-FTIR and SEM for the analysis of the human dentin was performed.

### 2.2. Fourier Transform Infrared Spectroscopy (ATR-FTIR)

All samples were previously evaluated, and the group irradiated subjected to ATR-FTIR analysis again after gamma irradiation. The samples were evaluated by ATR-FTIR, at a resolution of 4.0 $cm^{-1}$, with 100 scans, the absorption bands considered for this study were phosphate (1300-900$cm^{-1}$) and amide I (1680-1600$cm^{-1}$). The spectral data are truncated between 400 to 1800 $cm^{-1}$ for further linear baseline correction, and vector normalized.

### 2.3. Scanning Electron Microscopy (SEM)

For the analysis of dentin surface images, we used the Scanning Electron Microscope (Hitachi, model TM3000, Japan) where the images are generated by electron beams incidence on the surface of the sample. In the case of this equipment, a part of the primary electrons can be retained in the atoms of the sample thus, displacing electrons of the orbit of those atoms that would be discarded and generating secondary electrons. It is these secondary electrons that are responsible for generating the image in the SEM. These lenses may be magnified from 10 X to 200,000 X, the most frequently used being 10 X to 5000 X or 10,000 X. Three magnifications were selected for the specimens of this test as follows: 500 X; 1,000 X and 2,000 X.

## 3. RESULTS

### 3.1. FTIR

The Fig.1 shows the control and irradiated group dentin spectra. In the irradiated group, the band selected as representative of the inorganic content phosphate band (1300-900 $cm^{-1}$) shows an increase in the intensity and were not homogeneous. The band selected as representative of the organic content Amide I (1680-1600 $cm^{-1}$) showed an increase in intensity.

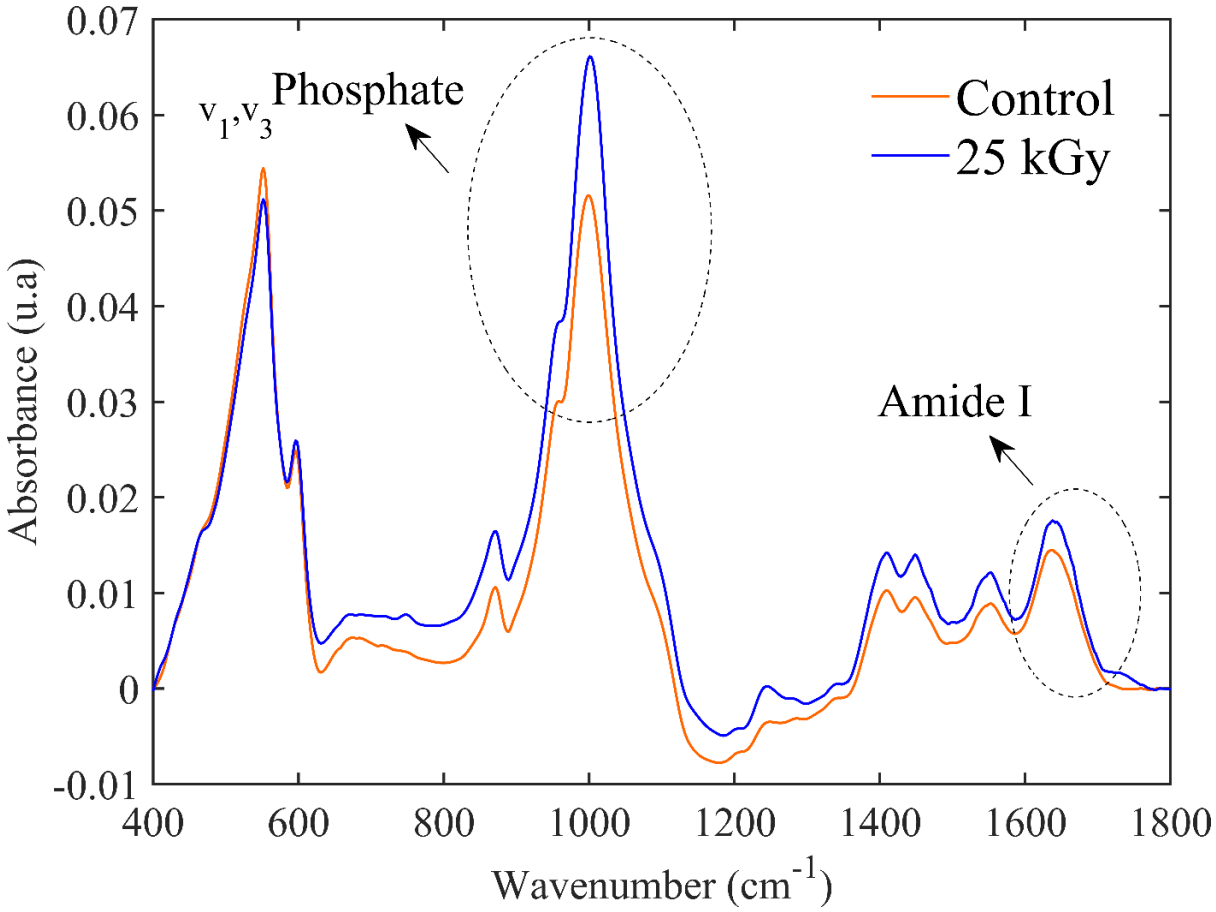


**Figure 1: Dentin Spectra Control and Gamma Irradiated Group.**

### 3.2. SEM

In the images obtained by SEM, shown in Fig. 2 and Fig. 3, it is possible to observe differences in the organization of peritubular dentin, intertubular dentin, and dentinal tubules. It is possible to observe a reduction of the present spaces in the intertubular and peritubular dentin in the irradiated group, due to the gamma irradiation. The shape in which the dentinal tubules appear in the irradiated group shows a decrease in the diameter of the dentin tubules compared to the control group, besides presenting an elliptical shape in comparison to the control group. It is possible to observe a morphological alteration of the dentine structure, suggesting cracks and

destruction.

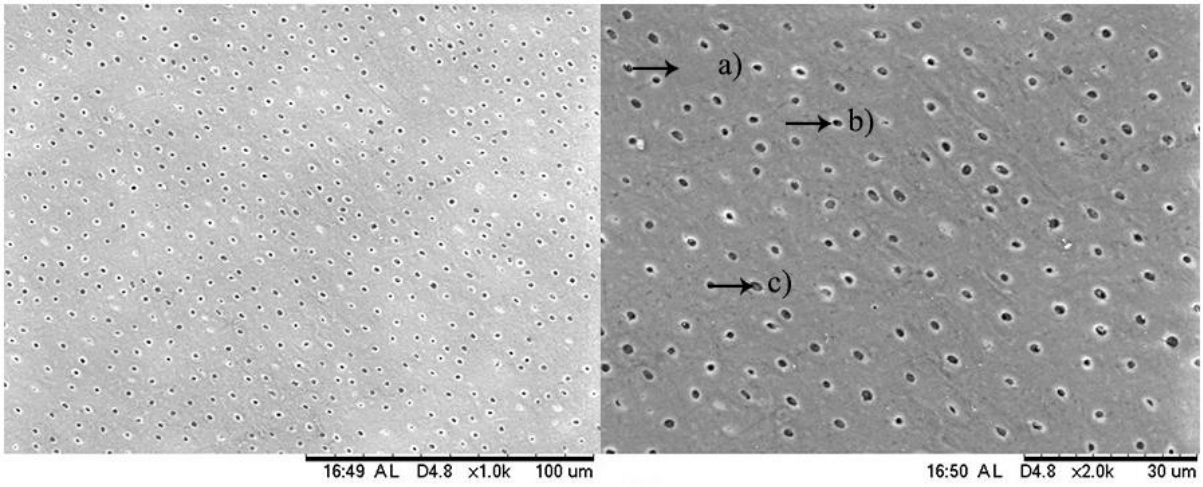


**Figure 2: Human Dentin Control Group a) Peritubular dentin. b) Intertubular dentin. c) Dentinal tubules.**

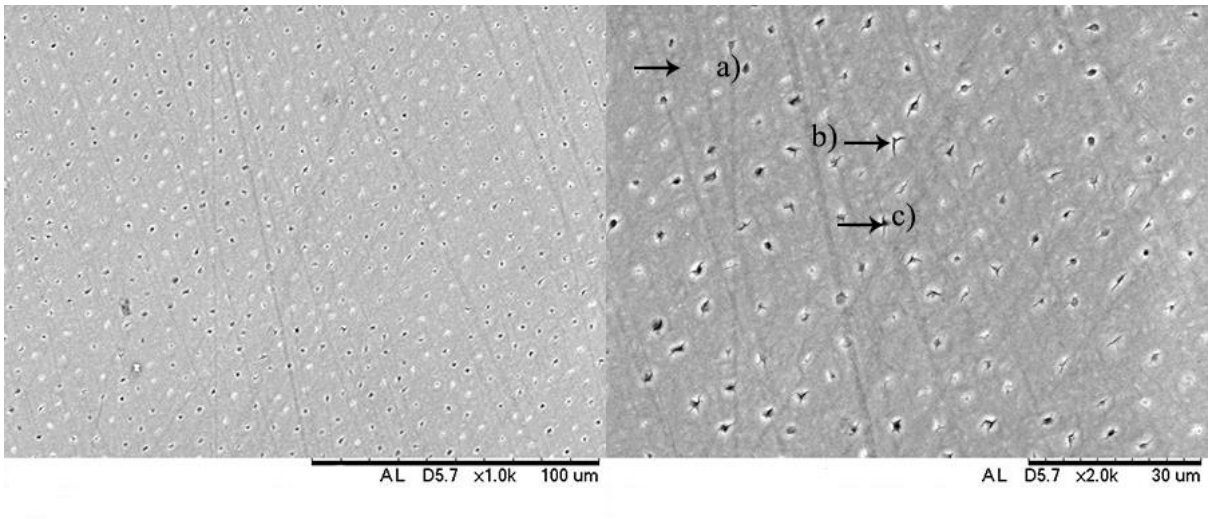


**Figure 3: Human Dentin Gamma Irradiated Group. a) Peritubular dentin. b) Intertubular dentin. c) Dentinal tubules.**

## 4. DISCUSSION

Dentin has approximately 70% of its mineral content in the form of hydroxyapatite, being characterized as vascular, avascular connective tissue, where odontoblastic extensions are present in the structure of the dentinal tubules, 18% are composed of mineral content and 12% water, more water than enamel series (2%). In conjunction with the dental pulp, dentin is a functional unit, called the dentin-pulp complex [5, 9]. As seen, the so-called caries of radiation primarily affect the region of dentin [2]. Thus, the biophysical impacts that happen through gamma radiation interaction with biological tissues need to be understood. The interaction of the ionizing radiation can interact with other ions that influence, change or modify them, the

higher quantity of organic material and water in the dentin must be taken into consideration [10, 11].

An important biophysical process of interaction with ionizing radiation is that of water radiolysis, where changes in the composition and energy levels of the molecules present will occur. When the ionizing radiation interacts with the water molecules, these molecules can be in an excited state, forming other unstable radicals of the type $H3O^{+}$, $H2O^{+,}$ and $H2O^{-2}$. These free radicals are very reactive and have no electric charge, which is why they can interfere with the metabolism of proteins, lipids, and carbohydrates, and as seen in the biochemical process of dental demineralization, increase hydrogen ions in the oral cavity. These reduce the pH of the buccal medium by binding to the $PO_4^{3-}$ ions forming phosphoric acid. Thus, causing the demineralization of the tooth and causing the radiation effect itself to alter the biochemical process of the oral cavity, in addition to the effects for the years after radiotherapy. These findings corroborate with other studies and also suggest degradation of the organic material, indicating that the gamma radiation changes the properties of the dentin, since it is a dental tissue with a greater amount of water [10-14].

FTIR analysis showed the presence of the absorption bands corresponding to the region of organic and the inorganic content of the tooth. Amide I band is the band of protein, primarily by the stretching vibrations of the C=O (70%-85%) e C-N (10%-20%). This analysis showed a significant difference in Amide I, suggesting an alteration of the organic content. This modification could have occurred as an effect of water radiolysis, increasing the chances of phosphoric acid formation in the oral cavity. Additionally, no important changes were discovered in the inorganic material represented by the phosphate band, corroborating with other research where the dental enamel stayed intact after gamma irradiation dosage protocols [7, 10, 12].

SEM images also show the dentin changes after gamma irradiation. Irradiated group presents irregular dentinal tubules and showed a destruction of intertubular dentin, this morphological analysis shows that the dentinal tubules did not collapse as occurs in conventional caries, a presence tubules in elliptic form, suggest the effect of the water radiolysis and heating during the gamma irradiation cycles and a degradation of collagen fibers, corroborating with the FTIR analysis.

## 5. CONCLUSIONS

The FTIR analysis, associated with the SEM analysis, corroborates with other studies confirming that the main modification that occurs in human dentin after exposure to gamma radiation, even at doses used much higher than the those used in radiotherapy protocols, is in the representative band of organic material. This suggests an alteration of the organic material by the effect of water radiolysis, with no significant alteration in the representative band of inorganic material, confirming the resistance of the hydroxyapatite of dental enamel exposed to gamma radiation even in doses of 25 kGy.

## ACKNOWLEDGMENTS

CNPq-INCT 465763/2014-6, CNPQ PQ 309902/2017-7; CAPES/PROCAD 88881.068505/2014-01, CNEN Edital PD 2017, FAPESP/CEPID 05/51689-2 and FAPESP 17/50332-0.

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