Molecular analysis of human and bovine hydroxyapatite from dental enamel and dentin submitted to gamma radiation

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Abstract: The important alteration of gamma irradiated hydroxyapatite in the buccal cavity depends on the biochemical alteration of the environment. The hydroxyapatite analyzed isolated showed a minimal molecular change of the crystal.

Introduction

The *in situ* model, at first proposed by Kolourides e Volker in 1964 and later modified by Zero in 1992 [1], consists in the applied research of the role of foods and fluoride in the processes of de-remineralization in humans without causing any interference in theirs natural dentition. When Zero, in 1995 [2], proposes a long-term *in situ* model, in order to allow an in vivo formation of a multicellular plaque. Concerning the substrate of samples used at the *in situ* model, Ghaeth and collaborators in 2011 [3], realized a literature review comparing the use of bovine teeth with human teeth at *in vitro* and *in situ* essays. There is a concern related in the literature regarding the bovine samples to stratify, prevent or minimize the risk of transmission of cattle diseases to the humans. Among the pathologies, Bovine Spongiform Encephalopathy (BSE) is the most worrisome. BSE emerged from the recent discovery of Creutzfeld-Jacob disease in humans and has been linked to transmission between species. The stratification of the risk of contamination of humans, from a bovine sample of known traceable origin, is not always possible and more efficient sterilization methods can be more aggressive to the enamel and dentin, also leading to changes at the biofilm formed *in vivo* at the long-term in situ model experiments. However, bovine bone graft has been widely used and considered safe from the biological point of view for the human usage with the finality of surgical reparation of bone defects. There is a consensus that the gamma radiation dose between 15 and 25 kGy seems to be a safe dose to the maintenance of the biosecurity of the receptor and mechanical and biological proprieties of the bone graft.

Objective

This *in vitro* study aims verifying the molecular modification and structural variation of the crystallinity of human and bovine hydroxyapatite from dental enamel and dentin submitted to gamma radiation.

Material and Methods

After the approval of the Committee of Ethics in Research, 15 samples of bovine dental enamel and 35 samples of human dentin of uniform dimensions were gamma irradiated. The evaluation of the initial surface microhardness of each polished sample was performed to allow the subsequent calculation of the percentage of surface microhardness loss (%SMHL) after sterilization by gamma radiation. A constant load of 50 gf was applied perpendicularly on the surface of the sample during 5 seconds by means of a microdurometer (Shimadzu HMV-2000, Japan) fitted with pyramidal diamond. Indentations were performed point by point in increasing distances from the selected border. Final indentations were performed following the same protocol. The set of samples was kept refrigerated during the gamma irradiation. Two dosimeters (Perspex Dosimeter, Harwell, UK) and a biological marker for sterilization were added at the set of samples. For irradiation, a Co^{60} multipurpose irradiator was used by means of the product overlapping source irradiation system. The irradiation time of the samples was approximately 2 hours making a total of 2 irradiation cycles of 12.5 kGy / cycle. The irradiated samples were submitted to structural integrity, molecular analyses and were evaluated morphologically by scanning electron microscope (Hitachi TM 3000, Japan). The molecular analysis of the hydroxyapatite was carried out by Fourier transform infrared spectroscopy (FTIR) Infrared absorption spectra were obtained with a spectrophotometer (Nicolet 6700, Thermo Scientific R) coupled to an attenuated total reflection accessory, ATR (Smart Orbit, Thermo Scientific R). The spectra were obtained with a resolution of 4 cm^{-1} and each spectra consist of an average

of 150 scans. These spectra were intervals of 4000-400 cm^{-1} (medium infrared) using a source emitting in the Globar type infrared and a DTGS detector (deuterated triglycine sulphate). The final mineral content was analysed by calculating the percentage of surface microhardness loss (% PDS) and ionisable Calcium concentration by a ion specific electrode (Orion, USA).

Results

The mean of the initial MDS was performed and the percentage of surface microhardness loss (% PDS) on enamel samples was peer-reviewed from the selected border. The determination of the % PDS after gamma irradiation is an intra-sample control, assessing the safety of the method used in relation to the maintenance of homogeneity. The dispersion of % PDS of the enamel samples subjected to sterilization by gamma radiation ranged from -0,0008<0,004. The morphological analysis by SEM showed mainly that the dental surface was heated in the proposed irradiation parameters. FTIR from enamel samples has shown that the molecular structure from gamma irradiated hydroxyapatite *in vitro* is similar to non irradiated human dental enamel with no formation or loss of molecular compounds, accordingly to figure 1. The alkali soluble Calcium from the dentin samples was statistically higher ($p<0.05$) in the non irradiated samples.

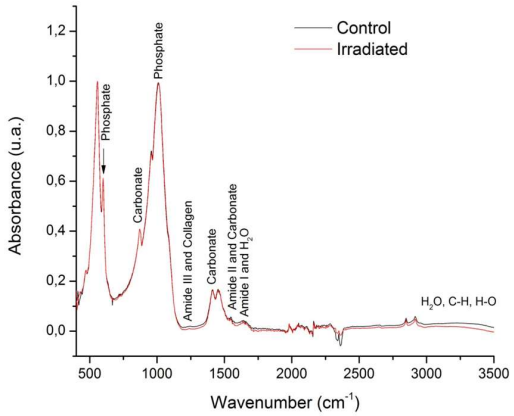

Figure 1 – The FTIR spectra show that gamma irradiated *in vitro* hydroxyapatite is similar to non irradiated hydroxyapatite originated from human dental enamel.

Conclusion

Gamma radiation applied to human dental enamel sterilization purposes seems to be safe and causes minimal changes at the mineral content and morphological aspects of enamel samples.

References

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