Journal of Oral Science, Vol. 46, No. 4, 215-220, 2004

Original

The effect of gamma radiation on enamel hardness and its resistance to demineralization *in vitro*

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(Received 20 February and accepted 8 September 2004)

Abstract: Given the importance of sterilizing human teeth before using them in research, the effects of a 25 kGy dose of gamma radiation on the mechanical properties of enamel and its resistance to demineralization were evaluated. Thirty human teeth were sectioned longitudinally, and while one half of each tooth was irradiated, the other half was kept as a control. Abraded and unabraded enamel slabs were obtained from these halves. The surface microhardness (SMH) of abraded slabs of irradiated and non-irradiated enamel was determined to evaluate the effect of radiation on enamel structure. Further, both abraded and unabraded slabs, either irradiated or non-irradiated, were submitted to a pH-cycling model to evaluate enamel resistance to demineralization, which was quantified by mineral loss (ΔZ) using cross-sectional microhardness. The data for SMH and ΔZ were statistically analyzed by t-test and ANOVA, respectively. The difference in enamel SMH between slabs from irradiated teeth and non-irradiated teeth was not statistically significant ($P > 0.05$). The abraded enamel slabs showed higher values of ΔZ than unabraded enamel slabs ($P < 0.05$), but the irradiation effect was not statistically significant ($P > 0.05$). The results suggest that the medical gamma radiation dose of 25 kGy does not affect either enamel hardness or its resistance to demineralization. (J. Oral Sci. 46, 215-220, 2004)

Key words: gamma radiation; sterilization; enamel; microhardness; demineralization

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Introduction

Disease transmission has long been a concern in the practice of medicine and dentistry. Some potential infection sources such as saliva, blood and body fluids are present in the clinical setting and, consequently, they can be present in extracted stored teeth. Such sources can cause hand, instrument or other material contamination enabling microorganism transmission to researchers and students who work with stored teeth (1). Although there have been no reports of disease transmission via extracted teeth, teeth used in teaching and research laboratories should undergo sterilization procedures.

The Center for Disease Control and Prevention (CDC) recommends that extracted teeth used for education and research purposes should be disinfected with sodium hypochlorite or liquid chemical germicides (2). However, sodium hypochlorite can increase the porosity of human enamel by deproteinization (3,4). The American Dental Association and CDC suggest autoclaving as the best sterilization method for materials exposed to body fluids (5). However, teeth can be damaged or altered by the sterilization process in an autoclave (6,7). Additionally, extracted teeth with amalgam restorations should not be

autoclaved because of the mercury vapor released in the air (1).

Another sterilization method considered effective is the use of ethylene oxide. However, White and Hays (8) have demonstrated the inefficacy of this gas against *Bacillus subtilis* spores placed in the pulp chamber of extracted human molars, so it is recommended that other methods should be used.

The authors believe that exposure to gamma radiation is another important and suitable method for dental sterilization, but its effect on enamel surface microhardness and on resistance to demineralization is not well known. This ionizing radiation produced by cobalt-60 is lethal to microbial populations and has high penetrative power (9). Gamma radiation does not cause morphological changes in the enamel surface (10-12). However, this method can alter the enamel color and its resistance to acid demineralization (11-14). These effects have only been tested with *in vitro* static models of caries production (12-14), which did not simulate the dynamics of caries development in the oral environment (15).

The gamma radiation dose of 25 kGy is the standard commonly used for sterilization of hospital supplies (16). Furthermore, it is the dose most frequently used in relation to tooth sterilization in the scientific literature (11-13,17,18). However, scientific data are not conclusive with regard to the effects of this dose on enamel structure and on its resistance to demineralization *in vitro* in conditions mimicking the caries process.

Consequently, this research aimed to verify the effect of a 25 kGy gamma radiation dose on human dental enamel surface microhardness, as well as analyzing the resistance of the irradiated enamel to demineralization when submitted to a pH-cycling model.

Materials and Methods

Experimental design

Thirty impacted human third molars were hemisectioned longitudinally, with both halves kept together throughout the experiments until being assigned to either control or irradiated groups. After irradiation, 60 enamel slabs were obtained from the non-irradiated and irradiated halves. The surfaces of 30 slabs, either irradiated or not, were abraded and polished. Four groups were formed with ten enamel slabs per group and submitted to a pH-cycling model: unabraded control, unabraded irradiated, abraded control and abraded irradiated. For the abraded groups, five slabs with mean hardness numbers higher than the mean value of each group, and five slabs with mean hardness numbers lower than the mean value of each group were chosen, resulting in two homogeneous groups (343.4 ± 6.15 and 345.4 ± 6.58). In order to assure the equality of the means of the newly formed group and in order not to benefit any group, the hardness values were statistically examined using a paired t-test, at 5% level of significance. There was no significant difference between the groups ($P = 0.601$).

The slabs (n = 20) of the unabraded groups were selected at random, using the lottery method (19). After pH cycling, enamel mineral loss was assessed by evaluating cross-sectional microhardness.

Tooth preparation and irradiation

The third molars used were stored in sterilized saline solution, had more than two-thirds of formed root, and were free from apparent caries, macroscopic cracks, abrasions and staining, as assessed by visual examination. They were longitudinally hemisectioned under aseptic conditions, with the operator wearing gown, gloves and mask to avoid contamination.

After sectioning, each group of 30 halves was immersed in 50 mL of sterile deionized distilled water. The experimental group was irradiated at the Agricultural Nuclear Energy Center-University of São Paulo with gamma radiation from cobalt-60. The irradiation was performed using a GAMMACELL 220 EXCEL (GC-220E) for 14 hours and 49 minutes at 27˚C producing a dose of 25 kGy. The irradiation time was determined taking into consideration the correction for radioactive decay of the gamma-ray source.

Mechanical property analysis

After the irradiation, 60 enamel slabs (4 × 4 × 2 mm) were obtained from each group of dental halves using a water-cooled diamond saw and a cutting machine. The enamel surfaces of 30 of the slabs were serially polished to undergo the surface microhardness test, while the other slabs were kept to be used in the pH cycling. Five indentations 100 μm apart were made at the center of each enamel slab. The surface microhardness from the abraded groups was measured using a microhardness tester with a Knoop diamond under a 25-g load for 5 s.

Demineralization-remineralization cycling (pH cycling)

Adhesive tape with a circular hole 2.0 mm in diameter was attached to the center of the enamel slab. The remaining surfaces of the slab were painted with acid-resistant nail varnish, so that a 3.14 mm^2 surface area was exposed.

The groups were subjected to five demineralization-remineralization cycles at 37˚C using the model originally described by Featherstone et al. (20) modified by Argenta et al. (21). Each cycle consisted of a 3-hour immersion in

demineralizing solution followed by a 21-hour immersion in remineralizing solution. The demineralizing solution was composed of 0.75 mM acetate buffer, containing 2.2 mM calcium ($CaCl_2$), 2.2 mM phosphate (NaH_2PO_4) and 0.03 µg F/ml. The pH of the solution was 4.3 and it was applied in the proportion of 6.36 ml/mm^2 of exposed enamel area. The chemical composition of the remineralizing solution was 1.5 mM calcium, 0.9 mM phosphate, 0.15 M KCl, 0.05 µg F/ml and 20 mM cacodylate buffer, with a pH of 7.0, applied in the proportion of 3.18 ml/mm^2. Both solutions contained thymol crystals to avoid microbial growth.

Cross-sectional microhardness analysis

Each enamel slab was longitudinally sectioned with a cut through the center of the exposed area. The segments were embedded in acrylic resin and the cut sections were polished. Three lanes of eight indentations each were made at the depths: 10, 30, 50, 70, 90, 110, 200 and 300 µm from the outer enamel surface in the central region of the dental slab, using a Knoop diamond under a 25 g load for 5 s. The distance between the lanes was 100 µm (Fig. 1).

Knoop hardness values (KHN) were converted to volume percentage mineral according to Featherstone et al. (22): volume % mineral = 4.3 KHN$^{1/2}$ + 11.3. After calculating volume percentage mineral values for each depth evaluated, mineral profiles, integrated area of mineral content and mineral loss values (ΔZ) were obtained for all groups (23,24).

Statistical analysis

The data were evaluated using the Shapiro-Wilks test to check the equality of variances and normal distribution of errors. The SMH data were analyzed with the paired Student's *t*-test. The ΔZ data were transformed by the power 0.3 and a two-way analysis of variance (ANOVA) with interaction was used when $P < 0.05$, to determine the significance of the irradiation, the enamel surface flattening and the interaction between these two factors. The difference between treatments was assessed by Tukey's test. The SAS software system (version 8.02, SAS Institute, Cary: NC, 1999) was used and the significance limit was set at 5%.

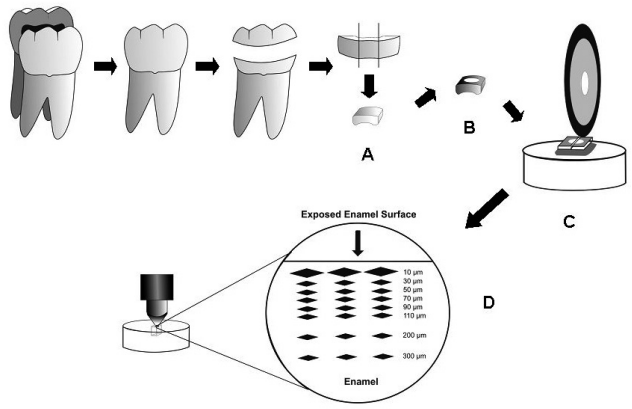

Fig. 1 **A** Human enamel slab obtainment. **B** Prepared and demineralized slab. **C** The enamel slab was sectioned in the central area. **D** Scheme of cross-sectional microhardness measurements.

Results

It was observed that the irradiation changed the color of dental structures from white to a cream color. This effect was observed in all samples, by comparing the tooth half from the control group with the corresponding irradiated tooth half.

However, a dose of 25 kGy did not change the mechanical properties of the enamel (Table 1), since the difference in enamel surface microhardness between the groups was not statistically significant ($P = 0.58$).

Table 2 shows the statistical description analysis of enamel mineral loss (ΔZ) according to the treatments/groups, after pH cycling. A two-way ANOVA demonstrated that the effect of the irradiation was non-significant ($P = 0.914$), as was the interaction between the irradiation and enamel surface flattening ($P = 0.057$), however, the effect of the enamel surface flattening was

Table 1 Means (± SD; n = 30) of enamel surface microhardness (kg/mm^2) of slabs from irradiated teeth and non-irradiated teeth (control)

Groups	kg/mm^2	*P**
Control	338.35 ± 20.11	0.58
Irradiated	340.77 ± 21.68	

*Difference not statistically significant (*t*-test)

Table 2 Means (± SD; n = 10) of enamel mineral loss (ΔZ) with regard to treatment/groups

Treatment/Groups	ΔZ (% mineral vol × µm)
Unabraded control	547.9 ± 135.9
Unabraded irradiated	425.0 ± 171.9
Abraded control	909.5 ± 405.5
Abraded irradiated	1106.5 ± 343.3

statistically significant ($P = 0.0001$).

Discussion

The present study showed by visual analysis that the gamma radiation changed the dental color. This result is consistent with previous studies carried out by Amaechi et al. (12-14). These authors attributed the color change to denaturation of organic components of the dental substrates.

The lack of effect of the 25 kGy gamma radiation dose on the microhardness of human teeth noted in the present study is in agreement with the results reported by Chandler (11) for bovine abraded slabs. In addition, studies carried out by Wieman et al. (25), Zach et al. (26) and Jansma et al. (10) did not find inorganic changes in enamel structure irradiated by ionizing radiation. Moreover, Jansma et al. (27) showed that irradiation did not affect enamel permeability. This suggests that proteic alteration could be the main reason for the dental color change, since the dentin has more proteins in its composition than the enamel and it is the substrate responsible for the tooth color. The more pronounced effect of gamma radiation on dentin was demostrated by Kielbassa et al. (28) who showed that dentin was severely altered by even lower irradiation doses.

The behavior of the irradiated enamel was similar to that found in previous studies performed by Walker (29), Amaechi et al. (12,14) and Kielbassa et al. (30). These studies showed no significant differences in enamel resistance to demineralization between irradiated and non-irradiated enamel, even though the latter study used a much lower gamma radiation dose. Other researchers have presented conflicting observations regarding the use of X-irradiation. An increase in resistance to demineralization was observed by Joyston-Bechal (31) and Jansma et al. (10), while a decrease in enamel resistance was reported by Jervøe (32), although this study did not entail any demineralization experiments. The author simply speculated that the structural change observed in the hydroxyapatite crystals of the irradiated enamel and dentin would increase the dental solubility.

With regard to the flattening of the enamel surface, one explanation for the increased mineral loss found in the abraded enamel slabs is the existence of a gradient in solubility or rate of dissolution of the enamel. Several studies, using an acid-etch technique, have shown that the rate of dissolution of human enamel mineral increased from the surface inwards (33-36). This gradient has been attributed to the higher mineral content in the outer enamel, as well as the concentration and distribution of some trace elements present in the enamel. The mineral content, in general, decreases from the tissue surface toward the dentin, while porosity, fluid, and organic material increase in this direction (37). Another factor that could be responsible for the high mineral content of the enamel surface is the presence of an aprismatic zone at the enamel surface, a feature which has been reported by many researchers (38-42). This layer is generally more mineralized than the enamel subsurface, because of the parallel nature of the crystals and the resultant lack of prism boundaries (43).

The fluoride content is concentrated very much at the enamel surface, and declines toward the tissue interior (44-46). The formation of a more stable fluoridated mineral at the enamel surface would effectively increase the surface caries resistance, since this ion stabilizes the apatite crystal structure (37) and interferes physicochemically with caries development by reducing demineralization and enhancing remineralization (47). On the other hand, carbonate concentrations rise from the enamel surface towards the dentin (48,49). This may explain the increased demineralization of the inner enamel layers, because the carbonate fits less well in the lattice, generating a less stable and more acid-soluble apatite phase (37,50).

Conclusion

A single 25 kGy gamma radiation dose does not seem to have an effect on the enamel surface microhardness or its resistance to demineralization.

The medical dose of gamma radiation (25 kGy) could be used to sterilize teeth because it does not change either enamel hardness or its resistance to demineralization.

Acknowledgments

This study was supported by CAPES (PICDT), from which the first author received a fellowship. The authors wish to thank Dr Cínthia P M Tabchoury and Dr Rosane M O Argenta, for their help with pH cycling. This publication was based on part of the thesis submitted by the first author to the Faculty of Dentistry of Piracicaba, UNICAMP, in partial fulfillment of the requirements for the master's degree in dentistry (cariology area).

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