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Effect of sterilization by gamma radiation on nano-mechanical properties of teeth

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SHORT TITLE

Mechanical properties of teeth after γ -irradiation

Key words

Tooth sterilization, gamma radiation; mechanical properties, atomic-force microscopy, nano-indentations, dentin, enamel

Abstract

Objectives. Extracted teeth used in dental research need to be considered infective and hence be sterilized without the materials properties being altered. This study examined the effect of gamma radiation on the nano-mechanical properties of dentin and enamel of extracted human third molars.

Methods. Whole teeth were sterilized using gamma radiation doses of 7 kGy and 35 kGy, respectively; teeth of the control group were not treated with gamma radiation. Crowns were sectioned occlusally and polished. Elastic modulus and hardness were tested using atomic force microscopy with nano-indentations under wet conditions.

Results. We found no significant dose-response relationship in elastic modulus or hardness in either dentin or enamel.

Significance. Nano-indentation is a common technique for the determination of local mechanical properties in biological hard tissues. Gamma radiation is an efficient way to sterilize extracted teeth while alterations of dentin and enamel mechanical properties are minimized.

1 Introduction

Development and testing of restorative dental materials use extracted teeth which are considered to be a potential biological hazard and source of bloodborne pathogens. Thus, infectious agents associated with extracted teeth need to be eliminated. In addition, minimal alterations of structure and properties of the tissue are desirable. In dental research, several sterilization methods are common including autoclaving, chemical heat or dry heat sterilization. However, they might either affect material properties of the teeth or they are unsuitable for amalgam-containing teeth [1].

Gamma radiation is an alternative sterilization method. It is commonly applied for sterilization of medical devices [2;3] and treatment of food [4;5]. In addition, it has successfully been applied for sterilization of bone allografts in orthopedic surgery [6;7]. However, the radiation doses used (25 to 40 kGy) often reduce the mechanical stability of the bone grafts which might cause the implant to fail [8-10].

Gamma radiation has been shown to sterilize non-carious extracted teeth effectively without affecting the material properties if radiation doses up to 2 kGy are used [11]. If carious teeth are used, higher radiation doses may be necessary and might cause alterations in material structure and properties. Nano-indentation has become a common technique for the determination of local mechanical properties of structural features in biological hard tissues [12-14]. Hence, while investigations of the mechanical properties (hardness, surface morphology and bond strength) on a micro-scale showed no effect of radiation doses up to 25 kGy [15-18], the effect on the mechanical properties on a nano-scale needed to be investigated.

This study examined the effect of gamma radiation on the nano-mechanical material properties of dentin and enamel of extracted non-carious teeth. We hypothesized that sterilization by gamma radiation in doses commonly used for laboratory applications does not affect the nano-mechanical properties of extracted teeth.

2 Materials and Methods

2.1 Tooth sterilization

Human third molars from subjects requiring extractions as part of dental treatment were used in this study. Teeth were collected following a protocol approved by the UCSF Committee on Human Research. Teeth were stored in Hank's Balanced Salt Solution (HBSS) after extraction. Two groups of teeth were sterilized by use of gamma radiation from a ^{137}Cs source for 40 hr (7 kGy) and 200 hr (35 kGy), respectively. A third group of teeth (the control group) were not treated with gamma radiation. In each group, six teeth were examined.

2.2 Sample preparation and nano-indentations

Crowns were sectioned occlusally using a Buehler Diamond Saw (Buehler, Lake Buff, IL) under running water. The specimens were polished initially with a series of SiC papers and then on polishing cloths with diamond suspensions down to 0.25 μm . All specimens were stored in HBSS to minimize surface demineralization [12]. Material properties were tested using AFM (atomic force microscopy) with nano-indentations; conducted under wet conditions. The AFM used in this study was a Nanoscope III (Digital Instruments/Veeco, Santa Barbara, CA) with the standard head replaced with a TriboScope indenter system (Hysitron Inc., Minneapolis, MN). A Berkovich Indenter (Hysitron Inc., Minneapolis, MN) was used for both imaging and indentation. Calibration was performed on a standard fused quartz sample with known elastic modulus.

Nano-indentations with a peak load of 400 μN on dentin and 2000 μN on enamel produced load-deformation curves, from which elastic modulus and hardness were calculated. The hardness, H , was calculated on the basis of maximum force divided by the projected contact area at maximum load, while the elastic modulus, E , was calculated from the contact stiffness, defined as the slope of the linear portion of the force/displacement curve during unloading near the maximum load. Indentations on intertubular dentin and enamel were performed at two different sites on both buccal and lingual sides with six indentations per site (*i.e.*, 24 indentations per tooth). Thus 18 teeth yielded 432 dentin and 432 enamel measurements of each outcome.

2.3 Statistical analysis

Separately for dentin and enamel, the outcomes were examined via graphical methods to identify skewness in the data. No transformations to achieve normality were required. Within tissue type (dentin or enamel), mixed-effects models were fit to determine the dependence of the outcomes on radiation dose (0, 7, or 35 kGy), while accounting for replicate measurements within teeth and sites per tooth. As an aid in identifying outliers, standardized residuals were examined via graphical methods to assess presence of outliers and poor model fit. In the enamel data, five datapoints with standardized residuals $> |3.4|$ were identified and excluded (3 were in the buccal location; all were at 35 kGy, Figure 1a). Dentin data were less variable: in models the outcomes as functions of location and dose, only 1 outlier was identified (residual = 3.15). However, in the model regressing elastic modulus on hardness two outliers were found (residuals, 3.4 and 3.7): one buccal at 35 kGy and one lingual at 7 kGy (Figure 1b). In keeping with the threshold used for enamel tissue, these were excluded from the analyses.

Although the purpose of this study was to determine the association between nano-mechanical properties and radiation dose, it was not possible to obtain longitudinal measurements of elastic modulus or hardness within teeth as whole teeth were irradiated but cut into slices and finished for indentations. To reflect periodic cross-sectional measurement of elastic modulus or hardness in distinct teeth, the significance of the association between mechanical properties and radiation dose was assessed via 2-df F statistics allowing for independent measurements across time. As we did not know whether radiation would increase or decrease the mechanical properties, or if such changes would be linear or nonlinear, we used the 2-df F statistic to examine each outcome as function of the dose categories (*i.e.*, a two-sided test that makes no shape assumption). Thus this statistic tests the null hypothesis of no difference among the mean outcomes against the alternative that some difference exists. Results are presented as mean and 95% confidence interval (CI).

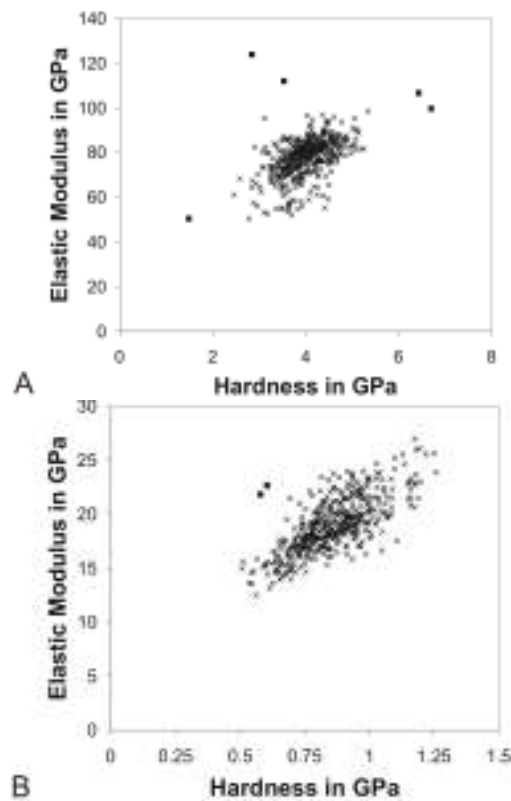


Fig. 1 Elastic modulus vs. hardness for enamel (top) and dentin (bottom) (■ outliers)

3 Results

To determine changes in nano-mechanical properties (elastic modulus and hardness) as a function of radiation dose, we first examined non-irradiated teeth used as baseline data (Table 1). Dentin of non-irradiated teeth showed an elastic modulus of 18.7 GPa (CI: 17.5 – 20.0 GPa) and hardness of 0.86 GPa (CI: 0.8 – 0.93 GPa). Elastic modulus of enamel was 77.2 GPa (CI: 72.2 – 82.2 GPa), hardness was 4.1 GPa (CI: 3.8 – 4.4 GPa).

Results of dose-response analysis for enamel (Table 1, Figure 2a) showed no statistically significant change with dose according to either elastic modulus or hardness: Hardness results showed a statistically nonsignificant ($p = 0.55$) decrease from 4.1 GPa (CI: 3.8 – 4.4 GPa) at 0 kGy to 4.0 GPa (CI: 3.7 – 4.3 GPa) at 7 kGy to 3.9 GPa (CI: 3.7 – 4.2 GPa) at 35 kGy. Elastic modulus of enamel also showed no trend ($p = 0.85$).

Table 1. Effects of radiation dose: results of trend analysis (mean and 95 % confidence interval)

Tissue	Dose kGy	Elastic Modulus GPa	Hardness GPa
Dentin (N = 430)	0	18.7 (17.5 – 20.0)	0.86 (0.80 – 0.93)
	7	18.7 (17.4 – 20.0)	0.85 (0.79 – 0.92)
	35	19.6 (18.3 – 20.9)	0.87 (0.80 – 0.93)
		$p = 0.51$	$p = 0.93$
Enamel (N = 427)	0	77.2 (72.2 – 82.2)	4.1 (3.8 – 4.4)
	7	78.9 (73.9 – 83.9)	4.0 (3.7 – 4.3)
	35	77.3 (72.3 – 82.3)	3.9 (3.7 – 4.2)
		$p = 0.85$	$p = 0.55$

Similarly, in dentin (Table 1, Figure 2b) only a statistically nonsignificant increase with radiation dose was observed for elastic modulus ($p = 0.51$): Values changed from 18.7 GPa at both 0 kGy (CI: 17.5 – 20.0 GPa) and 7 kGy (CI: 17.4 – 20.0 GPa) to 19.6 GPa at 35 kGy (CI: 18.3 – 20.9 GPa). Hardness values also showed now statistically significant trend ($p = 0.93$).

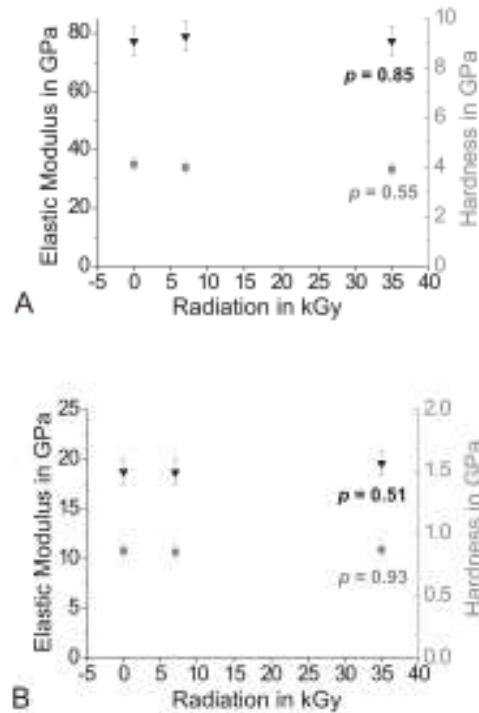


Fig. 2 Elastic modulus (\blacktriangledown) and hardness (\blacksquare , gray) vs. radiation dose for enamel (top) and dentin (bottom) (error bars: 95% confidence intervals)

4 Discussion

Gamma radiation can cause partial rupture of chemical bonds and reduction of specific ions. For inorganic materials, discoloration or fluorescence caused by introduction of defects caused by gamma radiation are well known [19;20]. While optical properties are affected by low radiation doses, the mechanical properties of inorganic materials are less easily affected. However, gamma radiation has been known to break chemical bonds of polymers including the peptide bonds of proteins. In synthetic polymers, gamma radiation can initiate oxidative aging by stripping of hydrogen from molecules and leaving free radicals [21]. Collagen consists of macromolecular chains of various kinds of amino acids. Its supercoiled triple helix conformation is sensitive to different degrees of radiation. Hence it has been suggested that it may be difficult to sterilize while maintaining the native structural integrity of the collagen fibers [22;23].

As enamel contains considerably less organic material than dentin we assumed that the mechanical properties of enamel would be less easily affected by gamma radiation than those of dentin. However, for the radiation doses used in this investigation, there was no effect on the nano-mechanical properties of dentin or enamel. The influence of the radiation dose on the nano-mechanical properties was investigated in trend analyses without making assumptions about the direction of the effects of radiation or the pattern of the association (*e.g.*, linear or nonlinear), thereby allowing the data to tell us what patterns exist, if any. Results for both elastic modulus and hardness of dentin and enamel (Table 1, Figure 2) showed no

changes with increasing dose ($p > 0.05$), even though the highest dose we examined is well above doses previously studied (35 kGy versus 25 kGy).

Enamel is the hardest and most highly mineralized substance of the body. 96% of enamel consists of mineral, with water and organic material composing the rest [24]. Dentin is made up of 70% inorganic materials, 20% organic materials, and 10% water by weight; 90% of the organic material is collagen type 1 and the remaining 10% ground substance which includes dentine-specific proteins. One possible explanation why the nano-mechanical properties of dentin were not affected by gamma radiation treatment is that while gamma radiation can break chemical bonds, it can also crosslink polymers. Hence breakage in peptide bonds of collagen may be compensated by the crosslinks formed [22]. Another possible explanation is that dentin collagen is reinforced by intrafibrillar mineral and each fibril is surrounded by extrafibrillar mineral. The intrafibrillar mineral, which stiffens the collagen fibrils, dominates the elastic behavior under normal loading conditions [25].

5 Conclusion

No significant association between nano-mechanical properties and gamma-radiation dose was found for either dentin or enamel of human third molars. Gamma radiation is a useful way to sterilize extracted teeth while alterations of the mechanical properties are minimized.

Acknowledgements

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