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Mechanical properties of human enamel as a function of age and location in the tooth

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Abstract Aging and the related changes in mechanical behavior of hard tissues of the human body are becoming increasingly important. In this study the influence of aging on the mechanical behavior of human enamel was evaluated using 3rd molars from young ($18 \leq$ age ≤ 30 years) and old ($55 \leq$ age) patients. The hardness and elastic modulus were quantified using nanoindentation as a function of distance from the Dentin–Enamel Junction (DEJ) and within three different regions of the crown (i.e. cervical, cuspal and inter-cuspal enamel). Results of the evaluation showed that the elastic modulus and hardness increased with distance from the DEJ in all three regions examined, regardless of patient age. The largest increases with distance from the DEJ occurred within the cervical region of the old enamel. Overall, the results showed that there were no age-dependent differences in properties of enamel near the DEJ. However, near the tooth's surface, both the hardness ($p < 0.025$) and elastic modulus ($p < 0.0001$) were significantly greater in the old enamel. At the surface of the tooth the average elastic modulus of "old" enamel was nearly 20% greater than that of enamel from the young patients.

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1 Introduction

Enamel is the hardest and most mineralized tissue of the human body. It is approximately 96% mineralized material by weight, with the remainder comprised of organic substance and water [1]. The mineral portion consists of carbonated hydroxyapatite crystals [2] that are tightly arranged together in prisms, with an effective diameter near 5 μm. Each prism is separated by a very thin (<<1 μm) layer of protein-based organic matrix [3, 4]. The prisms extend essentially perpendicular to the dentin–enamel junction (DEJ) and outward towards the tooth's surface. Within molars the thickness of enamel is largest near the cusps (up to approximately 2.5 mm), and then decreases to a minimum closest to the base of the crown [1].

Due to the limited volume of tissue available for examination, mechanical properties of human enamel have been primarily evaluated using indentation methods. Previous studies have found that the hardness ranges from approximately 3 GPa to 6 GPa and the elastic modulus ranges from 70 GPa to nearly 120 GPa [5–11]. Some of the property variations have been attributed to structural anisotropy that results from the enamel prisms and corresponding crystal orientation. In an examination of individual enamel rods, Habelitz et al. [8] found significant differences in the properties parallel and perpendicular to the prism; the lowest hardness and elastic modulus were obtained for indentations made perpendicular to the enamel rod axis. Ge et al. [12] attributed mechanical anisotropy to contributions of the more compliant interprismatic matrix, particularly for indentations made perpendicular to the enamel prism.

In addition to the importance of prism orientation, there are also spatial variations in the properties of enamel. Both the hardness and elastic modulus increase from the DEJ towards the tooth's surface. Cuy et al. [13] and Braly et al. [14] showed that the property distributions were related to the enamel chemistry and degree of mineralization. They deduced that the mechanical properties of enamel were primarily dependent on the extent of mineralization and that the effects of microstructure and orientation were secondary. Indeed, studies on hypomineralized enamel [15, 16] have shown that there are noticeable reductions in the hardness and elastic modulus of human enamel with relatively minor reductions in mineral content. Staines et al. [10] estimated that a 1% decrease in volume concentration of hydroxyapatite would result in a 3 GPa reduction in the elastic modulus.

There are natural changes in the mineral content of hard tissues with aging. For example, in human dentin the mineral content increases with patient age due to deposition of mineral salts within the tubule lumens [1]. This process has prompted studies focused on changes in the structure and chemistry of dentin, and their effects on the corresponding mechanical behavior [17–19]. In a comparison of dentin from young (22 ± 3 years) and old (61 ± 6 years) teeth, Senawongse et al. [20] found that the hardness and elastic modulus of dentin in the old teeth were larger, but that the changes were limited to a region just beneath the DEJ. Recent studies have shown that there is a significant reduction in the fatigue strength and fracture toughness of dentin with patient age [18, 21, 22], both of which increase the potential for tooth fracture.

In enamel, there are two relevant aging-related processes of importance. Specifically, there is a reduction in the proteinaceous matrix residing along the prism boundaries as a result of natural maturation and consumption of substances that lower the oral pH [1]. Also, prolonged exposure to mineral ions and fluoride within the oral environment can promote replacement of the matrix with fluoro-apatities [23], causing an increase in tissue density and a decrease in permeability [24]. Despite these aforementioned changes, no study has been reported that examined the influence of aging on the mechanical properties of human enamel. Therefore, the objective of the present study was to determine if the mechanical properties of enamel are dependent on patient age and if there are unique property changes within specific regions of the tooth.

2 Materials and methods

Human third molars were obtained from participating clinics within the state of Maryland according to an approved protocol issued by the Institutional Review Board of the University of Maryland, Baltimore County. Both the age and gender of the patient were obtained with each tooth. The teeth were placed in Hank's balanced salt solution (HBSS) immediately after extraction to minimize changes in properties with storage [25]. At receipt the molars were divided by age into "young" ($18 \leq$age ≤ 30; $N = 7$) and "old" ($55 \leq$ age; $N = 7$) age groups, which were defined according to results from previous evaluations on human dentin [21, 22] and not according to expected changes in properties. The average age and standard deviation of the young and old groups were 23 ± 4 and 73 ± 15 years, respectively. Fully erupted 3rd molars were used to avoid the influence of cuspal wear on the enamel thickness, and the potential for differences in properties due to large loads transmitted near the cusps.

The teeth were cast in a polyester resin foundation and sectioned using a programmable slicer/grinder[1] with diamond impregnated slicing wheels (#320 mesh abrasives) and continuous water-based coolant. A single longitudinal slice was made in the bucco-lingual plane approximately equidistant from the mesial and distal surfaces. One of the two halves was then mounted in a cold-cured epoxy resin and then polished using silicon carbide abrasive paper with successively smaller particle sizes. Further polishing was performed using diamond particle suspensions (Buehler) of sizes 9, 3, and 0.04 μm with a standard cloth wheel. The average surface roughness (Ra) resulting from the preparation was characterized after polishing using scanning probe microscopy (SPM) in contact mode. The surface roughness was measured over a single selected area of $50 \times 50\ \mu m^2$ in selected specimens and found to be 0.01 ± 0.003 μm. The sections were then bonded to a ferro-magnetic base using a cyanoacrylate adhesive for mounting the specimen to the nanoindenter stage. The specimens were maintained at room temperature (22 °C) in HBSS until evaluation and the surfaces were hydrated during testing.

Indentations were introduced in the prepared enamel surfaces using an automated nanoindenter[2] and a Berkovich diamond indenter with a 50 nm tip radius. A standard load/unload procedure was used with a rate of loading and

[1] K.O. Lee Model S3818EL, Aberdeen, SD.

[2] Hysitron Triboindenter, Minneapolis, MN.

unloading of 1 mN/s, and a maximum load of 5 mN held for 5 s. At this indentation load the average depth and edge length of the indentations were approximately 190 nm and 2 μm, respectively. Both properties were evaluated as a function of distance from the DEJ along six different paths and within three different regions of the tooth (Fig. 1a). Indentations were made on each sectioned surface along the buccal and lingual aspects of the cervical regions (A, F), at the buccal and lingual cusps (B, E), and within the inter-cuspal region (C, D). By virtue of the sectioning process and specimen orientation the indentations were essentially perpendicular to the enamel prism axis. However, as the prisms are rarely directed entirely straight from the DEJ to the outer surface, there is potential for small errors (i.e. ±10°) in the relative orientation between the indentation and prism axis; a recent evaluation of enamel using nanoindenation has shown that the effects of misorientation on the measurements would be minimal [14]. All six paths (A–F) were defined parallel to the enamel prism, beginning at the DEJ and continuing to the outer surface of the tooth. Indents were introduced at nine different equidistant sites along each path (Fig. 1b) and four indents were introduced at each site according to a square array with 20-μm center-to-center distance. The first four indents were placed approximately 20 μm from the DEJ, followed by increments of equidistant spacing over the defined path, and the last set of four indents was placed within 50 μm of the outer surface of the tooth. The distances from the DEJ to enamel surface in the cervical (A, F: ≈0.5 mm), cuspal (B, E: ≈2 mm) and inter-cuspal regions (C, D: ≈1.5 mm) were quite different. Therefore, the incremental distance between the nine sites ranged from less than 100 to over 200 μm apart and depended on the enamel thickness within that region. Through the aforementioned procedure, 36 indents were made along each path and a total of over 200 indents were introduced in the enamel of each tooth.

The hardness and elastic modulus were computed for every indentation using the traditional approach, which has been discussed in detail elsewhere [26]. Briefly, the hardness was determined from the ratio of applied load and indentation surface area and the elastic modulus was calculated using the stiffness of the unloading portion of the indent routine. It is important to highlight that the modulus reported herein is often regarded as the "reduced" elastic modulus [26]. Calibration of the Berkovich indenter was performed to obtain the tip area function using a fused quartz crystal. Due to differences in distance from the DEJ to the tooth's outer surface within the three regions of evaluation, the spatial property distributions were evaluated as a function of absolute distance, and also as a function of normalized distance from the DEJ. The normalized distance was established by dividing the distance of measurement from the DEJ by the total distance from the DEJ to enamel surface along that path of evaluation. Normalization enabled the property distributions to be compared objectively over a distance ranging from 0 to 1 along each path of evaluation, and for each tooth, despite differences in the enamel thickness within the three unique regions.

Using cumulative results for the teeth in each age group, the average hardness and elastic modulus of the young and old enamel were determined. The property distributions were quantified for each age group as a function of absolute and normalized distance from the DEJ including all paths, as well as separately for the three specific regions of evaluation (cervical, cuspal, and inter-cuspal regions). A comparison of these distributions was conducted within each age group and between the two age groups as well. Significant differences in properties at each measurement site were identified using an ANOVA ($p \leq 0.05$) and a comparison of property gradients was conducted using a two-sample Wilcoxon test ($p \leq 0.05$).

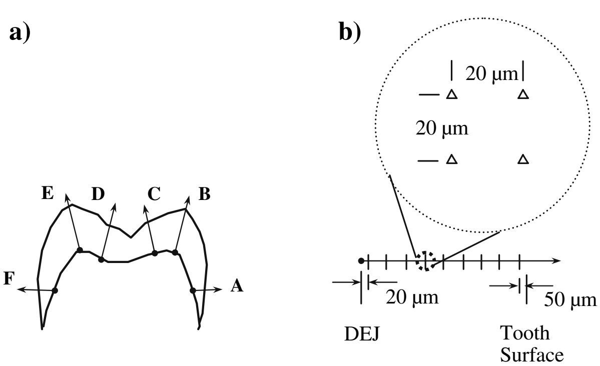

Fig. 1 Schematic diagram of a sectioned tooth and the six different paths of evaluation. Evaluation paths A and F (Cervical), B and E (Cusp) and C and D (Inter-Cuspal) were consistent for each of the 14 teeth evaluated. (**a**) Regions of evaluation and individual paths; (**b**) Nine locations of indents made along each path

3 Results

According to an assessment of all indentations performed, the average elastic modulus of enamel from the young and old molars was 84.4 ± 4.4 and 91.1 ± 6.5 GPa, respectively. Similarly, the average hardness of enamel from the young and old molars was 4.0 ± 0.3 and 4.0 ± 0.5 GPa, respectively. There was no significant difference ($p > 0.05$) between properties determined for the young and old age groups. In addition, the average elastic modulus and hardness of enamel within each of the three regions (i.e. cervical, cuspal and inter-cuspal regions) were not significantly different within or between the two age groups.

The elastic modulus distributions in the enamel of a young and old molar are shown in Fig. 2. Specifically, the elastic modulus distributions for a young and old molar are shown in terms of the absolute distance in Fig. 2a, b, respectively, and in terms of normalized distance from the DEJ in Fig. 2c, d, respectively. In general, there was an increase in the elastic modulus along all distinct paths (A–F), and for all teeth, regardless of age. However, the increase with absolute distance in the old molars appeared linear in all regions of the evaluation (Fig. 2b) in comparison to the largely non-linear distributions for the young enamel. Within the cervical, cuspal and inter-cuspal regions of the old enamel, the increase in elastic modulus with absolute distance from the DEJ was 15, 9 and 10 GPa/mm, respectively. While the increase within the cervical region of the old enamel was larger than that within the cuspal and inter-cuspal regions, the differences were not significant ($p = 0.273$).

Similar to the elastic modulus distributions presented in Fig. 2, the change in hardness with distance from the DEJ is shown for the selected teeth in Fig. 3. The variation in hardness with absolute distance from the DEJ is shown for a young and old molar in Fig. 3a, b, respectively. The hardness is presented for these two molars in terms of the normalized distance in Fig. 3c, d, respectively. Analogous with the trends in the elastic modulus, there was generally an increase in hardness with distance from the DEJ for all paths (A–F), and for all teeth. The increases in the old enamel were largest and primarily linearly distributed in comparison to those of the young enamel. Within the cervical, cuspal and inter-cuspal regions of old enamel, the increase in hardness with absolute distance from the DEJ was approximately 1.0, 0.7 and 0.7 GPa/mm, respectively. The differences in property gradients within these regions were not significant ($p = 0.095$).

As evident in Figs. 2 and 3, the properties of enamel within each of the three regions of evaluation exhibited consistent spatial distributions when examined in terms of normalized distance from the DEJ. Therefore, the properties obtained within each of the six unique paths of evaluation were combined for all teeth in each age group to obtain a cumulative description for the properties as a function distance from the DEJ. The average elastic modulus and hardness distributions for the young and old

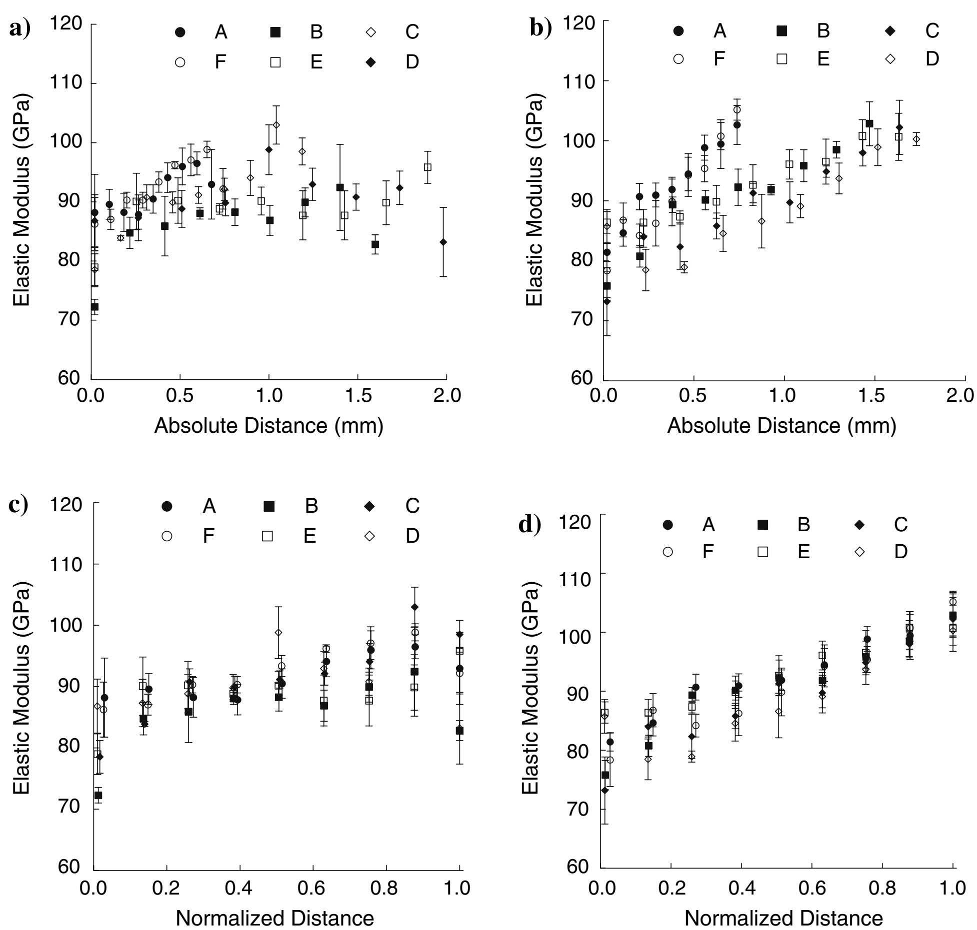

Fig. 2 The spatial distribution of the elastic modulus for enamel from selected young (22-year-old female) and old (57-year-old male) molars. The circular, square and diamond points correspond to properties within the cervical, cuspal and inter-cuspal regions, respectively. (**a**) Young enamel, absolute distribution. (**b**) Old enamel, absolute distribution. (**c**) Young enamel, normalized distribution. (**d**) Old enamel, normalized distribution

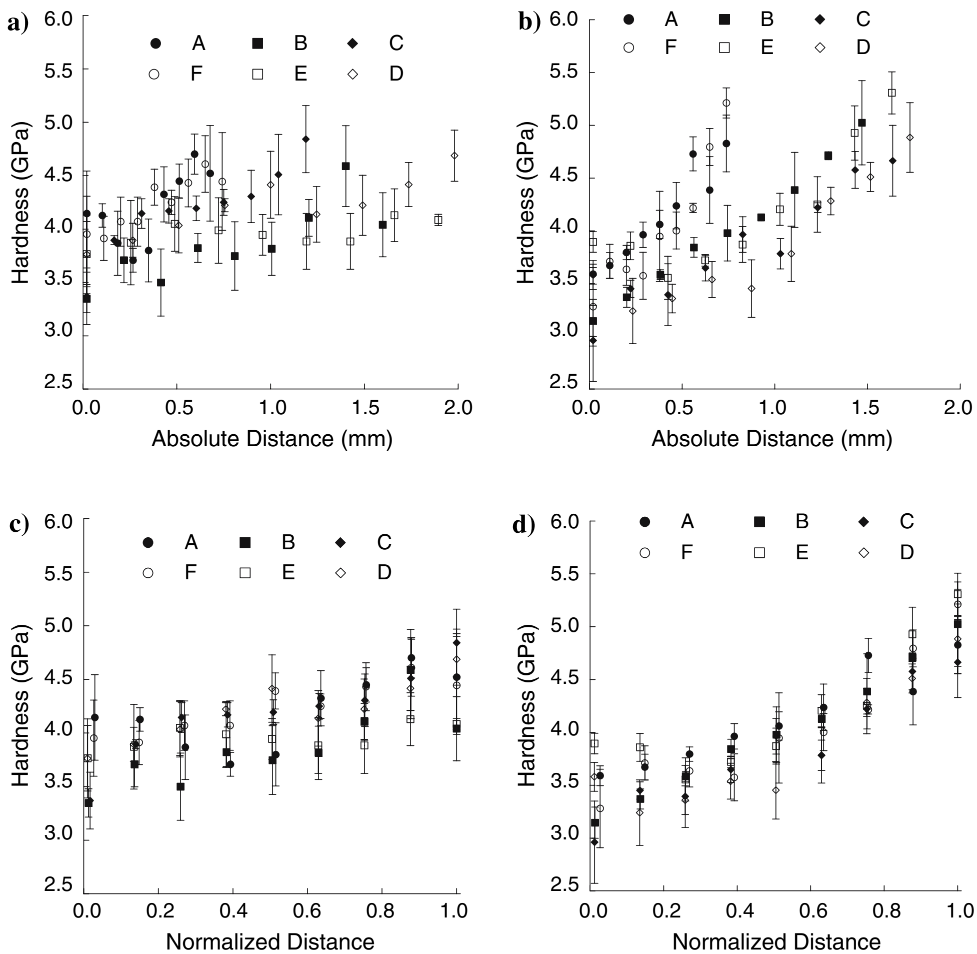

Fig. 3 The spatial distribution of the hardness for enamel from selected young (22-year-old female) and old (57-year-old male) molars. The circular, square and diamond points correspond to properties within the cervical, cuspal and inter-cuspal regions, respectively. The teeth selected are the same as those evaluated in Fig. 2. (**a**) Young enamel, absolute distribution. (**b**) Old enamel, absolute distribution. (**c**) Young enamel, normalized distribution. (**d**) Old enamel, normalized distribution

enamel are shown with respect to normalized distance in Fig. 4a, b, respectively. Note that the property descriptions presented in Fig. 4 are based on the results of all seven teeth for the respective groups. According to the ANOVA, the elastic modulus of old enamel was significantly greater ($0.0001 \leq p \leq 0.025$) than that of the young enamel; the level of significance increased with proximity to the tooth's surface. The old enamel was significantly harder than the young enamel at the tooth's surface only ($p < 0.025$). A power analysis was conducted with the elastic modulus and hardness data using means obtained for the young and old groups at each of the nine positions. When examining the elastic modulus, sufficient power existed for identifying significant differences at all nine positions of evaluation. For hardness, the difference in means relative to the variability showed that there was sufficient power to avoid type II errors at the tooth's surface only.

4 Discussion

Results obtained for the average elastic modulus and hardness of the young ($E = 84.4 \pm 4.4$ GPa, $H = 4.0 \pm 0.3$ GPa) enamel are in agreement with results of previous studies [5–11]. Also consistent with earlier investigations [5, 13], both properties increased with distance from the DEJ. Spatial variations in the properties of enamel could be attributed to a number of factors, the most likely of which are the potential differences in crystallography and chemistry. The hydroxyapatite crystals of outer enamel are considered more densely packed and tightly arranged than those within the inner enamel [27, 28]. Also, enamel exhibits tubules near the DEJ [29], which would reduce the effective volume fraction of mineralized tissue in this region and contribute to the comparatively lower hardness and elastic modulus. Yet, the largest contributions to spatial variations in properties are expected to come from the chemical composition and corresponding level of mineralization. There is a natural reduction in the interprismatic organic matrix with maturation of enamel. Prolonged exposure to fluoride in the oral environment results in a gradual increase in mineral content, particularly near the tooth's surface [30]. As such, the increase in hardness and elastic modulus of the enamel with distance from the DEJ is expected to result from the higher mineral content near the tooth's surface.

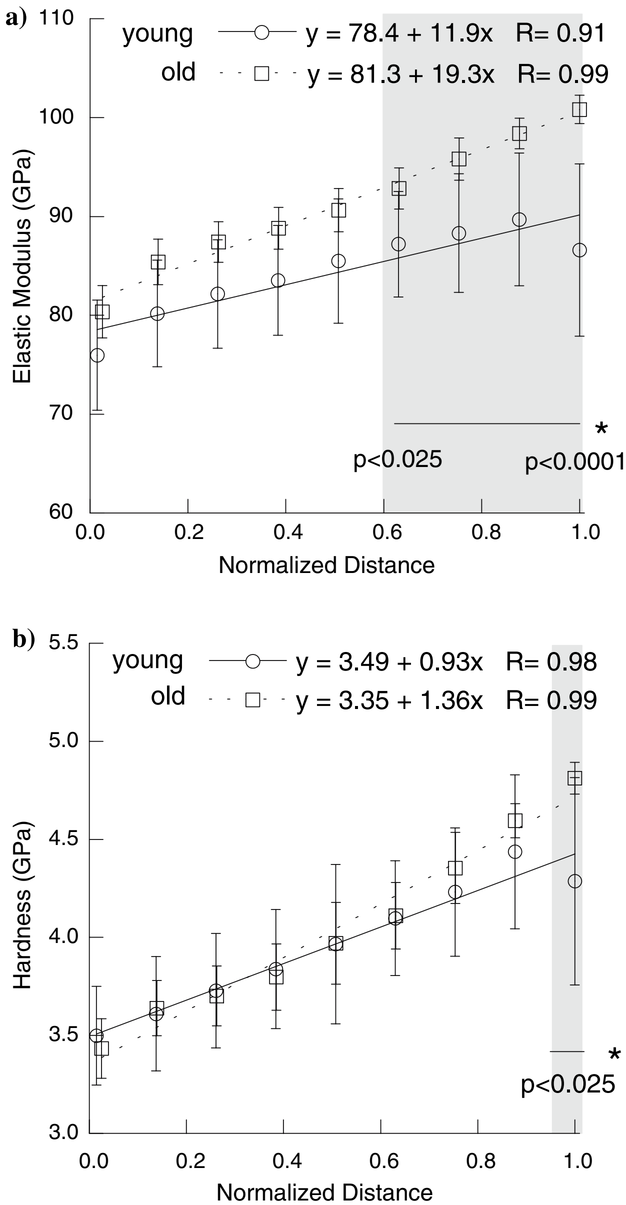

Fig. 4 Elastic modulus and hardness distributions of enamel for all teeth from the two age groups. *The highlighted area indicates a region of significant difference with level identified by the *p*-value. (**a**) Distribution in elastic modulus with normalized distance; (**b**) Distribution in hardness with normalized distance

While there was no significant difference in the average properties between the two age groups, there were significant differences between the properties of young and old enamel (Fig. 4) near the tooth's surface. No prior study has identified differences in properties of enamel related to patient age. Considering all three regions of evaluation (cervical, cuspal and inter-cuspal regions) the elastic modulus and hardness of the old enamel were 16 and 12% greater than those properties for young enamel at the tooth's surface. Surprisingly, the increase in these properties with age was also accompanied by a reduction in property variation. The coefficient of variation (COV) for the elastic modulus and hardness measurements are shown as a function of normalized distance from the DEJ in Fig. 5. Mechanical properties of the old enamel exhibited the lowest COV in all regions of evaluation. Furthermore, the COV for properties of the old enamel was lowest near the tooth's surface, whereas in young enamel the variation was highest at the tooth's surface.

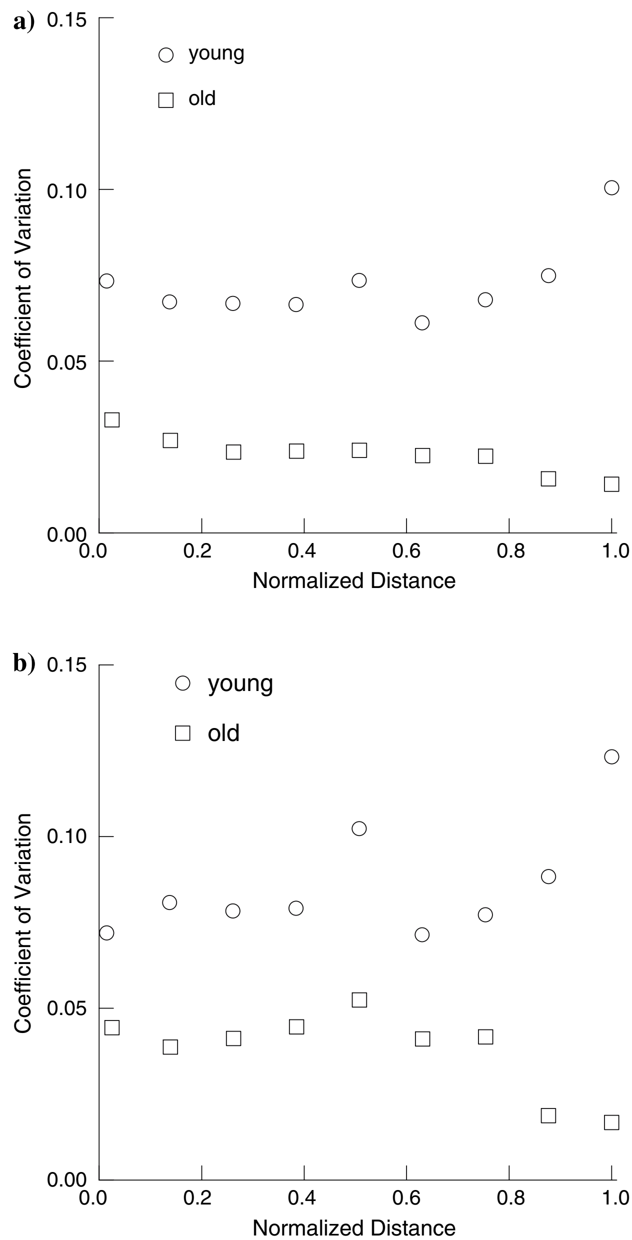

Fig. 5 Coefficient of variation for the mechanical properties as a function of normalized distance from the DEJ. (**a**) Variation in the elastic modulus; (**b**) Variation in the hardness

One of the most interesting aspects of property variations with normalized distance (Fig. 5) is the comparatively higher variation midway between the DEJ and tooth surface (normalized distance is 0.5) in the young enamel. The trend is most evident in the elastic modulus (Fig. 5a) and could result from the competing influences of two transport processes. Diffusion of mineral ions within enamel that originate from saliva undoubtedly decreases with increasing distance from the tooth's surface. As such, mineral changes would be expected to occur less rapidly within the inner enamel. But there is also potential for diffusion from the tooth's interior. Due to the positive pulpal pressure and additional driving force posed by the oral pH, there is potential for diffusion of mineral ions from dentin to enamel, especially in young teeth. Lynch and Ten Cate [31] recently found that remineralization of enamel lesions was assisted by diffusion of dissolved dentin mineral, and that the process was largely dependent on the relative distance between the enamel and dentin. Therefore, the difference in enamel thickness within the cervical, cuspal and inter-cuspal regions changes the relative contribution of the two modes of diffusion, and their potential for causing an increase in mineralization. Longer paths within the occlusal (B–E) regions would be less likely to exhibit uniform property changes with distance than shorter paths within the cervical region (A, F). Consequently, the largest variation in properties for the three regions of evaluation would be expected to appear midway between the DEJ and tooth's surface, and should be most evident when examined in terms of the normalized distance as identified in Fig. 5. In old enamel, the smallest property variations were evident at the tooth's surface. If the increase in properties of enamel result from a higher mineral content, then the combination of lower variation and significantly higher values at the tooth's surface suggests that the level of mineralization reaches a point of saturation after a particular age. Also of interest, according to the consistency in properties within the three regions, the saturation appears to occur uniformly across the entire crown of the tooth.

Though there were significant differences in properties of the young and old enamel, the importance of these differences and their relevance to restorative practices may not be readily apparent. It is expected that the increase in elastic modulus and hardness with age is at least partly associated with a reduction in the extent of interprismatic organic matrix. Previous studies have highlighted importance of the proteinaceous matrix on energy absorption, crack extension and the fracture toughness of this tissue [32–34]. In fact, White et al. [35] postulated that the larger fracture toughness of enamel in comparison to hydroxyapatite is associated with the unique mechanisms of toughening enabled by the organic matrix. If the increase in hardness and elastic modulus results from a reduction in the volume concentration of organic matrix, then there may also be a decrease in the fracture toughness of enamel with age. Moreover, for engineering ceramics, the material's brittleness is proportional to the hardness and elastic modulus, and inversely proportional to the square of the facture toughness [36]. Therefore, the rise in hardness and elastic modulus of old enamel at the tooth's surface could result in an increase in brittleness. Future work should be conducted to identify if there is a reduction in the fracture toughness of enamel with age due to loss of interprismatic organics [35], which would cause further increase in the brittleness. These studies are underway.

Results of the experimental investigation represent the first quantitative description for the changes in mechanical properties of human enamel with patient age. Nevertheless, there are recognized limitations to the investigation that warrant discussion. Of primary importance, the investigation did not couple measurements of the mechanical property distribution with a complementary examination of the enamel chemistry. Furthermore, the evaluation considered only two age groups and was limited to an examination of enamel from 3rd molars. The smaller COV for properties of the old enamel suggested that the properties reach a steady-state or saturation after a specific age. Future work should examine the properties over a more continuous age spectrum. Also, the age-dependent spatial variations could be influenced by the magnitude of occlusal forces, degree of wear and/or attrition. The properties of old enamel in teeth that play a larger role in oral functions could be unique from those described herein and should be examined. Lastly, recent evaluations have shown the importance of measurement parameters in quantifying properties of enamel using nanoindentation [37]. The indentation load and depth could influence contribution of the interprismatic matrix to the measured properties. Nevertheless, all the indentations in the present study were introduced using the same load, which provided an objective and consistent basis for comparison.

5 Conclusions

An evaluation of human enamel and the changes in mechanical properties with aging was conducted. The hardness and elastic modulus of enamel from fully erupted 3rd molars were examined using nanoindentation. The molars were divided into two groups corresponding to young ($18 \leq$ age ≤ 30) and old ($55 \leq$ age) patients. Properties were examined as a function of distance from DEJ and in three regions of the tooth (i.e. cervical, cuspal and inter-cuspal regions). The following conclusions were drawn:

1. The overall average elastic modulus of enamel from the young and old patients was 84.4 ± 4.4 and 91.1 ± 6.5 GPa, respectively. The overall average hardness from the young and old patients was 4.0 ± 0.3 and 4.0 ± 0.5 GPa, respectively.
2. The elastic modulus and hardness of enamel increased with distance from the DEJ for both age groups. When examined in terms of absolute distance from the DEJ, the gradient in hardness and elastic modulus was largest within the cervical region. However, when examined in terms of normalized distance from the DEJ, the properties distributions within each of the three regions were consistent.
3. The elastic modulus and hardness of the old enamel were 16 and 12% greater than those properties for young enamel at the tooth's surface. The differences in these properties between the two age groups were significantly different.

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