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Importance of Age on the Dynamic Mechanical Behavior of Intertubular and Peritubular Dentin

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Abstract

An experimental evaluation of human coronal dentin was performed using nanoscopic Dynamic Mechanical Analysis (nanoDMA). The primary objectives were to quantify any unique changes in mechanical behavior of intertubular and peritubular dentin with age, and to evaluate the microstructure and mechanical behavior of the mineral deposited within the lumens. Specimens of coronal dentin were evaluated by nanoDMA using single indents and in scanning mode via scanning probe microscopy. Results showed that there were no significant differences in the storage modulus or complex modulus between the two age groups (18–25 versus 54–83 yrs) for either the intertubular or peritubular tissue. However, there were significant differences in the dampening behavior between the young and old dentin, as represented in the loss modulus and $\tan\delta$ responses. For both the intertubular and peritubular components, the capacity for dampening was significantly lower in the old group. Scanning based nanoDMA showed that the tubules of old dentin exhibit a gradient in elastic behavior, with decrease in elastic modulus from the cuff to the center of tubules filled with newly deposited mineral.

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Keywords

Aging; Dentin; Dynamic Mechanical Analysis (DMA); Elastic Modulus; Nanoindentation

INTRODUCTION

Dentin occupies the majority of each tooth by both weight and volume, and exhibits a complex hierarchical structure that consists of both organic and inorganic components [1]. Mature dentin is composed of approximately 70% mineral (i.e. carbonated apatite), 20% organic materials (primarily type I collagen) and 10% water by weight [2]. The most distinct feature of its microstructure is the network of tubules that extend outward from the pulp towards the Dentin-Enamel Junction (DEJ). Each tubule lumen is surrounded by a peritubular cuff, which consists of a hyper-mineralized collagen-poor region (approx. 0.5 to 1 μm in thickness) of apatite crystals [1,3]. Intertubular dentin occupies the region between the tubules and consists of an organic matrix (collagen fibrils) reinforced by nanoscopic apatite crystals similar to that of peritubular dentin [4–6]. Due to the differences in composition between the peritubular and intertubular components, and striking presence of the dentin tubules in microscopic evaluations, dentin is often considered a biological composite.

There are two characteristics of human dentin that are clearly evident in microscopic evaluations of the microstructure. Firstly, there are spatial variations in the tubule density; the density decreases from roughly 60k lumens/ mm^2 near the pulp to 10k lumens/ mm^2 at the DEJ [7]. Secondly, the microstructure of human dentin is dependent on age of the individual. There is a reduction in the tubule lumen diameter (and increase in thickness of the peritubular cuffs) with increasing age. The process begins in the third decade of life and appears to progress at a constant rate until the complete occlusion of the lumens [8]. After the majority of tubule lumens are filled with mineral, the tissue appears translucent and is regarded as “sclerotic” [1]. As a result of the aforementioned process, human dentin undergoes an increase in mineralization with age [11–14]. This is directly opposite to that occurring in human cancellous bone, which undergoes a decrease in mineral content with age [9,10]. The comparison to cortical bone is more difficult as it undergoes an increase in mineral content with age during development to adulthood, and then either decreases with further aging [15] or develops a plateau [16]. Variations in findings appear to be associated with the exact location of bone being evaluated and what age range is being considered.

Owing to the spatial variations in microstructure of dentin within the tooth crown, the hardness [17], strength [18,19] and resistance to crack growth [20,21] all decrease with increasing proximity to the pulp. And according to the changes in microstructure of dentin with age, similar changes would be expected in its mechanical behavior. Indeed, there is a decrease in the strength of dentin with patient age. That process appears to begin near the end of the third decade of life, and proceeds at approximately 20 MPa per decade [22]. There is also a substantial reduction in the fatigue strength [13,20], fatigue crack growth resistance [23,24] and fracture toughness [25,26] with age. In comparing the fatigue crack

growth behavior, there is nearly a 100-fold increase in incremental growth rate within coronal dentin from young adulthood to an age of 50 years [22].

Spatial variations in strength and toughness of dentin do not appear to be reflected in the properties of the individual constituents. Kinney et al [27] reported that while the hardness of the intertubular dentin is substantially lower near the pulp than the DEJ, the hardness and elastic modulus of the peritubular dentin are essentially independent of depth. They attributed previous gradients in micro-hardness from the pulp to the DEJ to arise from the changes in intertubular dentin, not the tubule density. Nevertheless, that study was conducted on dehydrated tissue, and as expected, the indentation responses were found to be absent of viscous (i.e. dampening) behavior. Similar discrepancies are found in nanoscopic evaluations of the constituent properties and the influence of aging. For example, Balooch et al [28] evaluated the hardness and elastic modulus of the intertubular and peritubular dentin of normal and sclerotic tissue using atomic force microscopy. No significant difference was noted between the properties of normal and sclerotic tissue. Yet, Zheng and coworkers reported that the hardness of the sclerotic dentin beneath the cusps in old teeth was significantly greater than that of sound dentin from young teeth [29]. Similarly, Senawongse et al [30] found that 'aged' dentin exhibited higher hardness and elastic modulus than young dentin, but in the mantle region only (within 5 μm from the DEJ). The two latter studies were conducted on moist sections, but not hydrated, which could influence the capacity for dampening behavior. The aforementioned studies did not evaluate the properties of the peritubular and intertubular dentin independently. Thus, it is not possible to know which of the two constituents was responsible for the unique mechanical behavior of old dentin. Nanoscopic Dynamic Mechanical Analysis (nanoDMA) has been proven effective for characterizing the mechanical properties of hydrated dentin [31,32], but has not yet been applied to understand the effects of aging on the structural behavior.

In this investigation, nanoDMA was used to evaluate the mechanical behavior of human dentin, and quantify the changes that take place with aging. In the present study, the complex, storage and loss moduli were evaluated for the intertubular and peritubular dentin of teeth from a group of senior donors. These responses were compared to those from a similar evaluation of dentin that was conducted on a group of young donor teeth. The primary objective of the investigation was to identify if there are significant changes to the mechanical behavior of intertubular and peritubular dentin with age. The test null hypothesis was that there are no differences in the mechanical behavior of inter- and peritubular dentin with age.

MATERIALS AND METHODS

Human third molars were obtained from clinics within the state of Maryland according to a protocol approved by the institutional review board (IRB) of University of Maryland Baltimore County (Approval Y04DA23151). The teeth were stored immediately after extraction in 4°C Hank's Balanced Salt Solution (HBSS) to maintain the moisture content and prevent demineralization over the period of storage [33]. The donor age and gender were recorded for each tooth. A total of five teeth were obtained from donors aged 54 (Male), 62 (Female), 68 (Female), 70 (Male) and 83 (Female) years of age. They are

regarded as the “old” group. Results of the evaluation with these five teeth were compared with results obtained from a previous evaluation of “young” teeth [32] that included teeth from donors between 18 age 25 years. The young teeth were from donors aged 18 (Female), 19 (Male), 21 (Female), 24 (Female) and 25 (Male) years old. The age groups were defined according to previously reported findings on the changes in mechanical behavior of dentin with age [22].

Each tooth was encapsulated in polyester-based resin in order to provide a solid foundation for sample preparation. The encapsulated teeth were sectioned using a computer numerical controlled slicing/grinding machine (Chevalier, Smart H818-II) with a #320 mesh abrasive diamond slicing disk at 3000rpm. Serial sections were made perpendicular to the longitudinal axis of the tooth to obtain a single section of mid-coronal dentin approximately 2 mm thick. The slicing procedure was conducted with constant application of water spray to maintain hydration of the sample and to prevent elevations in specimen temperature during preparation.

The retrieved sections were mounted in cold-cured epoxy (Epofix HQ Resin and Hardener, Struers) according to the manufacturer’s recommendations. The samples were then polished under constant water irrigation to maintain hydration. Briefly, the polishing process began with #800 mesh abrasive paper to expose the section of dentin embedded within the resin mount. Thereafter, abrasive papers with mesh of #1200, #2400, and #4000 mesh were used successively to polish the surface. Finish polishing was performed using 3 μ m diamond particle solution, and 0.04 μ m colloidal silica suspension, with the corresponding polishing cloths recommended by the manufacturer (Struers).

The Dynamic Mechanical Analysis (DMA) was performed using a Hysitron TI 900 Triboindenter (Hysitron Inc., Minnesota MN). Both single indents and scanning-based evaluations were performed using a Berkovich diamond indenter with 100 nm nominal tip radius. During the indentation mode, single indents were introduced on either the intertubular dentin or the peritubular cuff. In this mode of evaluation the tip geometry and corresponding contact area was determined using the conventional approach with a fused silica standard sample [35]. For intertubular dentin, discrete indentations were made in locations that were at least 3 μ m from a peritubular cuff or previous indents. For the peritubular cuffs, only single indentations were performed on a cuff due to the limited cuff thickness. A visual survey of the indent was made after placement to confirm that the location was acceptable. All of the single indents were made using a static load with a superposed dynamic load applied at the peak indentation load. A static indentation load of 400 μ N and dynamic load amplitude of 20 μ N were used similar to those utilized in the study conducted by Ryou et al [32]. According to a parametric analysis performed in that investigation, a 400 μ N static load provides the highest sensitivity to the viscoelastic component of mechanical behavior during nanoDMA of dentin. The frequency of the superposed dynamic load was varied from 2 Hz to 100 Hz at ten different discrete frequencies, including 2, 4, 6, 8, 10, 20, 40, 60, 80 and 100 Hz. For the 60 Hz nominal frequency the testing was actually conducted at 58.5 Hz to avoid the introduction of signal artifacts after Ryou et al., [32]. In addition, testing at high frequency via nanoindentation can cause measurement artifacts associated with system resonance. To eliminate potential

influence from the frequency response of the test equipment the calibration procedure described in Hebert et al. [36] was implemented. This procedure measures the dynamic stiffness and damping of the TI-900 system over the selected testing frequency in air. Results from this calibration were then used to filter the system response. At least three indentations were made at each testing frequency, and on each constituent of the five different samples. Considering the 10 frequencies of evaluation, a total of 150 indentations ($3 \text{ indents} \times 10 \text{ frequencies} \times 5 \text{ samples} = 150$) were introduced and used in characterizing each of the intertubular dentin and the peritubular cuff.

The load and displacement signals captured during each indentation were used along with knowledge of the indentation area to estimate the storage (E') and loss moduli (E'') according to the methods for dynamic mechanical analysis after Odegard et al [34]. A detailed description of these methods for evaluating dentin is described in the study conducted by Ryou et al [32]. The indentation area (A) was estimated from the indentation depth using the tip area function, which was determined according to the conventional Oliver and Pharr approach [33]. Briefly, a series of indentations were introduced on a fused silica calibration sample (with known elastic modulus) at different indentation loads. The tip area function was generated over indentation depths from 50 nm to 260 nm, which spans the range of the indentations made in the intertubular and peritubular dentin. The machine compliance was calculated from the same data using the conventional approach described in the study conducted by Odegard et al [34].

The storage (E') and loss (E'') moduli represent the elastic behavior and dampening capacity of the material, respectively. These two parameters were used in estimating the complex modulus (E^*) according to $E^* = ((E')^2 + (E'')^2)^{1/2}$, which represents a measure of the combined influence of the storage and loss behavior [37]. The ratio of the loss and storage moduli were used to obtain the $\tan\delta$ parameter, which provides a relative measure of the viscous response of the tissue that is not dependent on tip geometry. A statistical analysis of the parameters was performed using a two-way Analysis of Variance (ANOVA) and Tukey's HSD test with significant differences identified at $\alpha = 0.05$.

In addition to performing nanoDMA of intertubular and peritubular dentin via discrete indents, a complimentary evaluation of the dynamic mechanical behavior was conducted using Scanning Probe Microscopy (SPM). Scanning based nanoDMA was performed using the same commercial instrument and Berkovich indenter as previously described. In this mode of evaluation the depth of indentation was very small and thus the actual tip geometry in contact with the substrate is potentially much different from the nominal geometry. Thus, the tip radius was determined by performing a scan on the fused silica standard sample and identifying the apparent radius needed for correct estimation of the reduced modulus; the apparent radius of the tip was approximately 800 nm. For the scanning evaluations a 4 μN static indentation load and a dynamic sinusoidal load of 2 μN were applied, with a dynamic frequency of 100 Hz after [38]. The scanning mode nanoDMA was conducted using an area of evaluation of $15 \mu\text{m} \times 15 \mu\text{m}$, which spanned a group of between 3 to 5 tubules. The instrument performs the evaluation through 256 horizontal scans, which are equidistant over the vertical dimensions of the image space. Over each horizontal scan path, 256 indents are performed by the instrument. Hence, each modulus map describing the nanoDMA behavior

in scanning mode is represented by an image with resolution of 256×256 pixels. Properties of the intertubular and peritubular dentin were evaluated by identifying regions within the data files that corresponded to the desired constituent. Then the average and standard deviation of the desired property were determined within that domain. In characterizing properties of the deposited mineral within the lumens of tissue from the “old” donors, the distribution in complex modulus was evaluated along radial line segments from the cuff/intertubular interface to the center of the newly mineralized region. The length of radial distance was then normalized, resulting in a length ranging from 0 to 1. Defining the scan length in that manner enabled a comparison of results amongst groups of tubules despite the differences in tubule dimensions.

To minimize the potential for changes in the mechanical behavior of dentin related to a variation in moisture content [39–42], both single indent and scanning modes of the nanoDMA evaluation were performed with hydration after the method of Ryou et al [38,43]. Briefly, a drop of 99.4% ethylene glycol was applied to the fully hydrated polished sample and distributed evenly across the surface using a cloth saturated with ethylene glycol. Ethylene glycol has been used previously to maintain the hydration of dentin samples during experimental evaluations as described by Pashley et al [44]. Application of the ethylene glycol film prevents the evaporation of water, and eliminates the problems related to the meniscus forces transferred from liquid droplets to the indenter. The method has been used to maintain sample hydration in nanoindentation studies of dentin [32], resin-infiltrated dentin [38] and adhesive interfaces involving dentin [43,45,46]. A validation study was performed prior to the studies on dentin via single indent and scanning mode evaluations on a fused silica sample. These activities were performed using the aforementioned conditions for indentation with and without the ethylene glycol treatment. Results showed that there was no influence of the ethylene glycol on the moduli estimates using either the conventional or DMA approaches [38].

The microstructure of selected specimens was evaluated using a Hitachi S-4700 (Hitachi High-Technologies Corporation, Tokyo, Japan) Field Emission Scanning Electron Microscopy (FE-SEM). Prior to evaluation, the samples were sputtered-coated with gold-palladium. Features of the microstructure were examined in both secondary electron and backscattered emission modes.

RESULTS

Micrographs documenting the microstructure of dentin from the two age groups are presented in Figure 1. As is evident in Figure 1(a), the majority of tubules in the old dentin were completely filled with mineral. A highly magnified view of a single tubule filled with mineral is shown in Figure 1(b). While not all tubules were completely filled, those that were filled did not show a transition from the original cuff to the newly mineralized region. For comparison, the tubules of dentin within the young group (Figure 1(c)) exhibited distinct lumens with an internal diameter of approximately 1 μm . A highly magnified view of a single tubule is shown in Figure 1(d). Apart from the lumen patency, there are no other apparent differences in the microstructure between the two age groups. The regions

corresponding to the peritubular cuff and intertubular dentin are clearly evident in the microstructure of both age groups.

The dynamic mechanical behavior of dentin from the “old” donor samples and the influence of loading frequency is shown in Figure 2. Specifically, Figure 2(a) shows the complex modulus of both the intertubular and peritubular dentin across the range of loading frequency performed. The complex modulus represents the indentation modulus including both elastic and dampening components. A separation of the elastic and dampening components is presented in terms of the storage and loss moduli in Figures 2(b) and 2(c), respectively. In addition, the $\tan\delta$ responses and their dependence on frequency are shown in Figure 2(d), which quantifies the relative degree of dampening behavior. In comparing responses obtained from the two constituents, the complex and storage moduli of the peritubular cuff were significantly greater than for the intertubular dentin ($p < 0.001$). The overall average complex modulus of old intertubular and peritubular dentin were approximately 21 ± 2 GPa and 31 ± 3 GPa, respectively; the average storage moduli for those regions were 20 ± 2 GPa and 30 ± 4 GPa, respectively. In contrast, there were no significant differences in the loss modulus and $\tan\delta$ parameters for the intertubular and peritubular components.

Results for single indent mode nanoDMA of the old dentin are shown in Figure 2. The property distributions in Figure 2 show that the loading frequency does not significantly influence the elastic component (i.e. storage modulus) of the dynamic mechanical responses for either the intertubular or peritubular dentin. The storage modulus increased roughly 11% over the 100-fold increase in frequency, from 19 GPa to 21 GPa. Similarly, the complex modulus in the intertubular dentin increased approximately 10% as well, from 19 GPa to 21 GPa. However, the dampening components of the responses (loss modulus and $\tan\delta$) underwent an increase with increasing frequency for both constituents. The loss modulus and $\tan\delta$ of the intertubular dentin both increased by approximately 300% from 0.4 GPa to 1.5 GPa and from 0.02 to 0.08, respectively. These two parameters increased as well for the peritubular dentin. The loss modulus and $\tan\delta$ of the peritubular dentin each increased by over 200%, from 0.8 GPa to 2.5 GPa and from 0.02 to 0.08, respectively.

Results obtained for the nanoDMA of the old dentin are compared with results reported for young dentin in the study conducted by Ryou et al [32] in Figures 3 and 4. Specifically, the frequency responses for the intertubular dentin of the two age groups are compared in Figure 3. That comparison for the peritubular dentin is shown in Figure 4. When comparing responses for the two age groups, neither the complex modulus (Figure 3a and Figure 4a, respectively) or storage modulus (Figure 3c and Figure 4c, respectively), are dependent on age for the intertubular and the peritubular dentin ($p > 0.05$). However, there are significant differences in the dampening behavior between the age groups. Both the loss modulus (Figure 3b and Figure 4b) and the $\tan\delta$ (Figure 3d and Figure 4d) responses of the old dentin are significantly lower than those quantities for the young dentin over a particular frequency range. That statement is true for loading frequency of 10 Hz and above in the responses obtained for both the intertubular and peritubular components.

The property distributions in Figures 3 and 4 show that the dynamic mechanical behavior of dentin is dependent on loading frequency in both age groups. However, the frequency response for tissue representing the two age groups is not equivalent. In young dentin, the capacity for dampening (expressed by the loss modulus and $\tan\delta$ response) continues to increase with loading frequency. The capacity for dampening is significantly greater ($p < 0.05$) for frequency greater than 20 Hz than at the lower frequencies. That behavior is reflected equally in the intertubular and peritubular dentin. For the old dentin, the capacity for dampening does not increase consistently with frequency. There is significantly greater dampening ($p < 0.05$) at higher frequency, but not until reaching a frequency of approximately 60 Hz and greater. There is similarity in the responses for both the intertubular and peritubular components of old dentin.

Results for scanning mode DMA obtained from a representative old dentin specimen are shown in Figure 5. Note that this figure presents maps for the complex, loss and storage moduli in Figures 5(a), 5(b), and 5(c), respectively. The profile height distribution is shown in Figure 5(d) and the $\tan\delta$ distribution is shown in Figure 5(e). Interestingly, the tubules are clearly visible amongst the dentin matrix as they were for all samples examined. Considering the entire scan window (both constituents) resulted in an average complex modulus of 20 ± 10 GPa (Figure 5a), loss modulus of 6 ± 6 GPa (Figure 5b) and storage modulus of 16 ± 7 GPa (Figure 5c); the average $\tan\delta$ was 0.5 ± 0.6 (Figure 5e). From an evaluation of the constituents individually, the intertubular possessed an average complex modulus of 18 ± 7 GPa, loss modulus of 4 ± 4 GPa and storage modulus of 15 ± 6 GPa; the average $\tan\delta$ was 0.4 ± 0.2 . For the peritubular dentin, the average complex, loss and storage modulus was 35 ± 18 GPa, 15 ± 11 GPa, and 26 ± 14 GPa, respectively. The $\tan\delta$ was 0.7 ± 0.6 . Results for the storage modulus of old dentin were not significantly different ($p > 0.05$) from those reported by Ryou et al [32] for young dentin in scanning mode. Surprisingly, there was also no significant difference in the parameters describing the dampening behavior between the two age groups that are tested with scanning mode nanoDMA.

Although the nanoDMA scan in Figure 5 was obtained in a region where the tubules were completely filled with minerals, there is a shadow of the lumens evident in the property maps. The lumens are clearly apparent in the complex, storage and loss moduli distributions, as well as in the surface topography. Hence, a comparison of the properties for the original cuff and the deposited mineral within the lumen was performed using the property maps obtained in scanning mode. Briefly, the distribution in properties was evaluated as a function of the radial distance from the apparent center of the cuff as shown in Figure 6(a). The radial profile in complex modulus of filled peritubular cuffs are shown in Figure 6(b). As evident from this figure, the complex modulus of the mineralized region at the center begins at approximately 22 GPa. There is an increase in modulus with radial distance until reaching a plateau of approximately 35 GPa. The plateau begins at approximately half the normalized radial distance (i.e. $r/R^* = 0.5$).

DISCUSSION

An experimental evaluation of human dentin was performed using nanoDMA to quantify the mechanical behavior and changes that occur as a result of biological aging. The importance

of age was assessed in terms of the dynamic moduli for the intertubular and peritubular components of coronal dentin from a group of “old” (54–83 years) donor teeth and from a comparison of those results with previous measures for “young” donor teeth (age 25). Results showed that while there was no apparent influence of age to the elastic component of the indentation response, there were significant differences in the dampening behavior. These results require rejection of the null hypothesis that there are no differences in the mechanical behavior of intertubular and peritubular dentin with age. There were also differences in the frequency response between the two groups. Both of these findings warrant discussion.

Due to the increase in mineral content of dentin with increasing age [11–14], changes to the mechanical behavior are not unexpected. Indeed, previous investigations have identified that there is an age-related reduction in the strength of dentin under both monotonic and cyclic loading [13,22], the fatigue crack growth resistance [23,24] and fracture toughness [13,25,26]. The reduction in strength begins near the end of the third decade of life and proceeds rather uniformly with increasing age [22]. Deposition of mineral within the lumens also begins in the third decade of life and progresses uniformly until the lumens have become completely filled [8]. Consequently, both the onset of degradation, and its rate of progression, would appear to coincide with the reduction in lumen diameter.

Previous investigations have adopted bulking loading and indentation approaches to evaluate the importance of aging to the elastic behavior and hardness. In studies performed using flexure, no significant differences were found between the elastic modulus of young and old dentin [22] or between that of normal and sclerotic tissue from aged teeth [13]. Similarly, no significant differences were found in the elastic modulus or hardness of intertubular dentin between normal and sclerotic tissue via atomic force microscopy [28]. Zheng and coworkers used microindentations to compare the trends in elastic modulus and hardness of tissue over an evaluation space extending from deep to peripheral dentin [29]. Although there was no significant difference in the elastic modulus from the two age groups, the hardness of old dentin was significantly greater than that for the younger tissue close to the DEJ. Senawongse et al [30] reported similar findings, i.e. within the mantle region the hardness and elastic modulus of old dentin were significantly greater than the values for young dentin. As the present evaluation was focused on mid-coronal dentin, the consistency in storage modulus for the two age groups (in Fig. 3(c) and Fig. 4(c)) agrees with these prior studies. Yet, comparing the findings across these studies suggests that the structural changes caused by biological aging are not homogeneous over the tooth crown. Furthermore, the nanoDMA shows that the dampening (or viscous) behavior of the tissue is much more sensitive to the structural changes that occur with aging (Fig. 3(b) and Fig. 4(b)) than the quasi-static behavior.

Nanoindentation has been recognized as a useful tool in the characterization of hard tissues [32,47–49]. The present investigation represents the first attempt to assess the time-dependent behavior of “old” dentin, and the material's ability to dissipate mechanical energy. In the application of nanoDMA, the material's capacity for dissipation of elastic energy is represented by the loss modulus (E''). The $\tan\delta$ value provides a relative measure of viscous dissipation from the ratio of storage and loss moduli [37]. Both the loss modulus and the

$\tan\delta$ values showed that the capacity for viscous deformation was significantly lower in the old dentin, and the reduction encompassed a relatively large range of dynamic loading frequency (Figures 3(b) and 4(b)). Specifically, above 10 Hz the viscous component of response for the old dentin was reduced to less than one quarter of that for the young tissue. One may question the relevance of this observation as routine mastication occurs at between 1 and 2 Hz [50]. Higher frequency oscillations (20 to 140 Hz) do occur during mastication and as a result of reflex reactions [76]. An increase in loading frequency is synonymous with an increase in loading rate, which is commonly encountered outside of routine mastication during impact events. Thus, the higher loading frequency used in nanoDMA has practical significance. Interestingly, Nalla et al [51] and Kruzic et al [52] observed frequency dependence in the fatigue life and fatigue-crack growth behavior of dentin, with greater fatigue resistance at higher frequency. In the nanoDMA responses for young dentin, the capacity for energy dissipation increased significantly with frequency (Figures 3(b) and 4(b)) and to a much greater extent than in the old dentin. An inability to dissipate energy at the nanoscale results in more energy available to activate mechanisms of energy dissipation at greater length scales (e.g. microcracking and crack growth). Hence, due to its reduced capacity for dissipating mechanical energy, old dentin is more prone to brittle fractures than young dentin under increasing rates of loading.

The nanoDMA analysis performed with discrete indents included isolated indentations of the intertubular dentin and peritubular cuffs. Consequently, the reductions in loss modulus and $\tan\delta$ of the old dentin were not directly attributed to the mineral filling the lumens. Dentin is a composite of collagen molecules and nanocrystals of hydroxyapatite. Akin to bone [53], it derives its mechanical behavior from the collective contributions of these elements and their structural arrangements over length scales ranging from the nano- to the micro-scale [13]. Aging of bone has a detrimental impact to the mechanical behavior, which is manifested through a reduction in strength and resistance to fracture [54,55]. Historically the age-related increase in bone fragility has been attributed to the decrease in “bone quantity”, i.e. via the reduction in bone mineral density [56–58]. Although there is also a decrease in collagen content with age, its contribution to the reduction in bone strength is far smaller than the degradation associated with bone mass [59]. There is now growing evidence that the reduction in strength and toughness of bone with age is related to degradation in “bone quality” [60,61]. Zimmerman et al [62] recently presented convincing evidence that this degradation takes place over multiple length scales and is largely attributed to specific changes in the bone collagen.

There is an increase in mineralization of dentin with age through the deposition of mineral within the lumens [11–13]; the mineral concentration of the intertubular dentin reportedly remains unchanged [11]. There is also a continual reduction in the pulp cavity due to the continual deposition of dentin [63]. Thus, there is an increase in “dentin quantity” with age. Though the most visible change in microstructure is the reduction in lumen diameter, the decrease in loss modulus of the old dentin could be attributed to changes in the dentin matrix.

In comparison to bone, the literature on dentin collagen is relatively scant. Owing to the similarity in composition and properties of these two tissues [64,65] a short discussion of the

changes in bone collagen with aging is relevant. Collagen plays an integral role on the mechanical behavior of bone [66,67]. In regards to aging, the collagen undergoes a process of maturation that involves, above all, a change in the degree of crosslinking. There are two types of collagen crosslinks in bone, namely enzymatic and nonenzymatic. Zioupos et al [68] showed that there is no apparent correlation between the enzymatic crosslinks and age or mechanical properties of bone. Nonenzymatic crosslink develop Advanced Glycation End products (AGEs) that establish both intra- and interfibrillar crosslinks [61]. Early experimental studies have identified that these nonenzymatic crosslinks cause a substantial reduction in the strength and toughness of bone [69,70]. Zimmerman et al [62] showed that the increase in AGEs stiffened the fibrils, which substantially decreases the capacity for inelastic deformation and intrinsic toughening in aged bone, both of which are critical to strength and toughness. Consistent with maturation of the bone matrix, Miura et al [71] recently identified that there is an accumulation of AGEs in dentin collagen with age. The AGEs accumulate within the crown and the root and were correlated with an increase in stiffness of the demineralized dentin matrix. In that study the density of AGEs appeared greatest within the collagen located nearby to the dentin tubules. Therefore, there is a degradation in the quality of human dentin with age. And if the nonenzymatic collagen crosslinking is responsible for an age-related degradation in strength and fracture resistance of human bone, an equivalent response is expected in dentin.

Akin to bone, human dentin undergoes a reduction in strength and toughness with aging. Yet, the measures of mechanical behavior using nanoDMA did not reflect the capacity for inelastic deformation. At the scale of the indentations, deformation within the dentin matrix consists of stretching of the collagen molecules and finite sliding as in bone [72]. This may also involve deformation of the mineral crystals located within the inter- and intra-fibrillar spaces and poroelastic process associated with water movement. Stiffening of the collagen fibrils through an increase in the degree of nonenzymatic crosslinks (AGEs) would reduce their capacity for stretching and sliding. Though potentially most relevant to the capacity for inelastic deformation, that would also diminish the components of finite deformation through reinforcement of the matrix. As such, the reduction in loss modulus and $\tan\delta$ values of the old intertubular dentin (Fig. 3(b) and 3(d)) could arise from the accumulation of AGEs with increasing age [71]. This mechanistic interpretation is admittedly speculative and requires further scrutiny. Another potential contribution, small angle X-ray scattering (SAXS) analysis of sclerotic dentin showed that the intertubular dentin of sclerotic tissue exhibits a lower average crystallite size (7~16%) in comparison to young dentin [14,73]. A reduction in crystal size or increase in packing density may contribute to the reduction in dissipative components of deformation as well.

The mechanisms contributing to the reduction in loss modulus and $\tan\delta$ of the peritubular dentin with age (Fig. 4(b) and 4(d)) are not clearly apparent. It is possible that the differences are associated with a reduction of the poroelastic processes related to water movement. Dissolution of mineral crystals within the intertubular dentin and re-precipitation within the peritubular cuff with age [14] would undoubtedly cause filling of the transverse secondary tubules and restrict water movement. X-ray diffraction measurements have shown that there is an increase in the Ca/P ratio with age that coincides with the progression of

mineral filling the lumen. This could lead to the precipitation of interstitial CaO crystals [13] and a densification of both the cuff and intertubular dentin as well.

Intertubular dentin is a three-phase composite consisting of a hydroxyapatite (HAP) component, a collagen fibril component, and fluid-filled component [74]. As intertubular dentin deforms, it is thought to transfer load from the more compliant fluid-filled collagen matrix to the reinforcing elastic HAP platelets. Mineralized peritubular dentin lacks a fluid-filled collagen matrix. The protein matrix of peritubular dentin is a mixture of acidic phosphoproteins (140–170kDa) that function much like phosphorin, but has a different amino acid composition [5] that is free of hydroxyproline. The major proteins of peritubular dentin seem to be developmental proteins, rather than structural proteins. They probably serve to control calcium binding and crystal growth.

The lower loss modulus in old peritubular dentin may be due to the loss of water from mineral occluded dentinal tubules that prevents stress-induced fluid shifts. As mineral crystals displace intratubular water, the peritubular dentin becomes progressively more dehydrated [75]. Both would serve to reduce the water content and its movement. Clearly these comments are speculative. Nevertheless, the consistency in loss modulus distribution of the peritubular and intertubular components with frequency (Fig. 2b) suggests that the mechanisms contributing to the changes in each component are coupled. Further work is necessary to isolate the mechanisms responsible for the decrease in relative viscous behavior of the peritubular dentin with aging. Those efforts should also extend over a larger range in strain to evaluate the difference in mechanisms of deformation within both elastic and inelastic regimes.

Results from the scanning mode nanoDMA evaluation of old dentin showed that there is a gradient in the stiffness from the center of the occluded lumen outward to the original cuff. Previous studies of old dentin [13,14] noted that there was a lower mineral density within the filled tubules of sclerotic dentin. That was also evident from the backscatter SEM images due to the sensitivity of backscattered electrons to mineral packing density variations. Additional high magnification images of single tubules from the old dentin samples are shown in Figure 8. A single tubule is shown in secondary electron and backscattered electron modes in Figures 8(a) and 8(b), respectively. From the backscatter electron images (e.g. Figure 8b) it was consistently noted there was lower grayscale intensity within the central portion of occluded lumens with respect to the adjacent peritubular cuff. That may be the cause for the gradient in complex modulus with radial distance and significantly lower values than that of the adjacent cuffs. There are also variations in the crystal size. Porter et al [14] showed that the mineral filling the tubule lumens consisted of plate-like crystals with much coarser grains than that of the cuff proper. Indeed, larger crystal sizes were evident in the lumens during the SEM analysis at high magnification (Figure 8(c)).

The results from single indent and scanning mode evaluations may appear inconsistent. Although the single indent mode showed that the degree of dampening in the responses for the young dentin was substantially larger than for the old dentin, results from scanning suggested there was no difference. This apparent discrepancy is easily addressed. Note in Figures 3(b) and 4(b) that the loss modulus for the young and old dentin are significantly

different over nearly the entire range of high frequency loading ($f = 10$ Hz). Yet, at 100 Hz the responses for the two age groups are actually equivalent. The scanning mode nanoDMA was performed at 100 Hz, which is the frequency where the young and old dentin did not exhibit significant differences. Although lower loading frequencies (lower than 100 Hz) were attempted in scanning mode, those lower than 100 Hz resulted in signaling artifacts in the scanned images. Thus, results for both scanning and discrete indent mode evaluations are in agreement, and the loading frequency is an important component to identifying age-related differences in mechanical behavior. Under the range of dynamic loading performed, a frequency of between 20 and 60 Hz resulted in the largest degree of difference between the young and old tissue.

Results from the nanoDMA showed that there is a significant reduction in the dampening behavior of human coronal dentin with age. Despite the importance of that finding, there were limitations to the investigation and important concerns. One limitation of the present study is that it was not spatially resolved, to enable an assessment of spatial variations in mineral to collagen ratio and its importance to the dynamic mechanical behavior. That is equally true for reported evaluations concerning changes in mineralization and matrix quality of dentin with aging. Future studies that address the structural changes and mechanical behavior of dentin should attempt to develop a spatially-resolved description of properties, which will help to develop a more robust mechanistic understanding of the aging process. A concern in most applications involving nanoindentation is the contribution of surface topography to the evaluated properties [e.g. 77,78]. Special precaution was taken during the discrete indentation mode of evaluation to avoid regions near the interface between the peritubular cuff and intertubular matrix and the edge of the lumens. Consequently, the surface topography was not an issue in the indentation mode. However, there was an influence of the topography on the scanning mode evaluations as evident in Figure 5 from the asymmetric properties around the tubules. Care was taken to avoid contributions from these artifacts in the measures of the cuff properties by excluding measures near interfaces when possible. An additional perceived limitation of the investigation is that the maximum loading frequency was limited to 100 Hz. It would be interesting to see if the dampening capacity of dentin continues to increase with frequency (Figure 2) at greater loading rates. Future work addressing this topic appears warranted.

CONCLUSIONS

On the basis of the results obtained, the following conclusions may be drawn:

1. For the selected group of old donor teeth evaluated, the average storage modulus of the intertubular dentin $18 \leq E' \leq 22$ GPa was significantly ($p < 0.05$) lower than that obtained for the peritubular dentin $28 \leq E' \leq 35$ GPa. The storage modulus of neither the intertubular or peritubular dentin was dependent on the dynamic loading frequency.
2. There was no significant difference in the loss modulus or $\tan\delta$ values between the intertubular and peritubular dentin of the old donor teeth. In addition, both of these parameters increased significantly ($p < 0.05$) with increasing loading frequency.

Over the frequency range from 1 to 100 Hz, the loss modulus for the peritubular and intertubular dentin increased by nearly 200% and 300%, respectively.

3. There was no significant difference ($p>0.05$) in the values of storage modulus obtained for the old dentin and that reported for young intertubular and peritubular dentin (donor age 24 years). However, both the loss modulus and $\tan\delta$ values for the old dentin were significantly lower ($p=0.05$) than those values for the young dentin over loading frequency from approximately 10 to 80 Hz.
4. The mineralized region at the center of the tubules that is deposited with the lumen during biological aging exhibits a lower complex modulus than the surrounding original peritubular cuff.

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References

1. Nanci, A. Ten Cate's Oral Histology: Development, Structure, and Function. 7. Saint Louis: Mosby Inc; 2008.
2. Summitt, JB.; dos Santos, J. Fundamentals of operative dentistry: a contemporary approach. Chicago: Quintessence Publication; 2006.
3. Xu C, Wang Y. Chemical composition and structure of peritubular and intertubular human dentine revisited. Arch Oral Biol. 2012; 57:383–391. [PubMed: 21996490]
4. Marshall GW, Marshall SJ, Kinney JH, Balooch M. The dentin substrate: structure and properties related to bonding. J Dent. 1997; 25:441–458. [PubMed: 9604576]
5. Weiner S, Veis A, Beniash E, Arad T, Dillon JW, Sabsay B, et al. Peritubular dentin formation: crystal organization and the macromolecular constituents in human teeth. J Struct Biol. 1999; 126:27–41. [PubMed: 10329486]
6. Kinney JH, Marshall SJ, Marshall GW. The mechanical properties of human dentin: a critical review and re-evaluation of the dental literature. Crit Rev Oral Biol Med. 2003; 14:13–29. [PubMed: 12764017]
7. Pashley DH. Dentin: a dynamic substrate—a review. Scanning Microsc. 1989; 3:161–74. [PubMed: 2662395]
8. Arola, DD. Fracture and aging of dentine. In: Curtis, R.; Watson, T., editors. Dental Biomaterials: Imaging, Testing and Modelling. Cambridge, UK: Woodhead Publishing; 2008. p. 314–342.
9. Smith D, Khairi M, Johnston C Jr. The loss of bone mineral with aging and its relationship to risk of fracture. J Clin Invest. 1975; 56:311–318. [PubMed: 1150873]
10. Kanis JA, Melton LJ, Christiansen C, Johnston CC, Khaltaev N. The diagnosis of osteoporosis. J Bone Miner Res. 1994; 9:1137–1141. [PubMed: 7976495]
11. Weber D. Human dentine sclerosis: a microradiographic survey. Arch Oral Biol. 1974; 19:163–169. [PubMed: 4527828]
12. Vasiliadis L, Darling A, Levers B. The histology of sclerotic human root dentine. Arch Oral Biol. 1983; 28:693–700. [PubMed: 6579901]
13. Kinney JH, Nalla RK, Pople JA, Breunig TM, Ritchie RO. Age-related transparent root dentin: mineral concentration, crystallite size, and mechanical properties. Biomaterials. 2005; 26:3363–3376. [PubMed: 15603832]
14. Porter AE, Nalla RK, Minor A, Jinschek JR, Kisielowski C, Radmilovic V, et al. A transmission electron microscopy study of mineralization in age-induced transparent dentin. Biomaterials. 2005; 26:7650–7660. [PubMed: 16005961]

15. Boskey AL, Coleman R. Aging and bone. *J Dent Res*. 2010; 89(12):1333–48. [PubMed: 20924069]
16. Akkus O, Polyakova-Akkus A, Adar F, Schaffler MB. Aging of microstructural compartments in human compact bone. *J Bone Miner Res*. 2003; 18(6):1012–9. [PubMed: 12817753]
17. Pashley D, Okabe A, Parham P. The relationship between dentin microhardness and tubule density. *Dent Traumatol*. 1985; 1:176–179.
18. Giannini M, Soares CJ, de Carvalho RM. Ultimate tensile strength of tooth structures. *Dent Mater*. 2004; 20:322–329. [PubMed: 15019445]
19. Ryou H, Amin N, Ross A, Eidelman N, Wang D, Romberg E, et al. Contributions of microstructure and chemical composition to the mechanical properties of dentin. *J Mater Sci: Mater Med*. 2011; 22:1127–1135. [PubMed: 21455677]
20. Ivancik J, Neerchal N, Romberg E, Arola DD. The reduction in fatigue crack growth resistance of dentin with depth. *J Dent Res*. 2011; 90:1031–1036. [PubMed: 21628640]
21. Ivancik J, Arola DD. The importance of microstructural variations on the fracture toughness of human dentin. *Biomaterials*. 2013; 34:864–874. [PubMed: 23131531]
22. Arola DD, Reprogl R. Effects of aging on the mechanical behavior of human dentin. *Biomaterials*. 2005; 26:4051–4061. [PubMed: 15626451]
23. Bajaj D, Sundaram N, Nazari A, Arola DD. Age, dehydration and fatigue crack growth in dentin. *Biomaterials*. 2006; 27:2507–2517. [PubMed: 16338002]
24. Ivancik J, Majd H, Bajaj D, Romberg E, Arola DD. Contributions of aging to the fatigue crack growth resistance of human dentin. *Acta Biomater*. 2012; 8:2737–2746. [PubMed: 22484693]
25. Koester KJ, Ager JW III, Ritchie RO. The effect of aging on crackgrowth resistance and toughening mechanisms in human dentin. *Biomaterials*. 2008; 29:1318–1328. [PubMed: 18164757]
26. Nazari A, Bajaj D, Zhang D, Romberg E, Arola DD. Aging and the reduction in fracture toughness of human dentin. *J Mech Behav Biomed Mater*. 2009; 2:550–559. [PubMed: 19627862]
27. Kinney JH, Balooch M, Marshall SJ, Marshall GW, Weihs T. Hardness and Young's modulus of human peritubular and intertubular dentine. *Arch Oral Biol*. 1996; 41:9–13. [PubMed: 8833584]
28. Balooch M, Demos S, Kinney JH, Marshall GW, Balooch G, Marshall SJ. Local mechanical and optical properties of normal and transparent root dentin. *J Mater Sci: Mater Med*. 2001; 12:507–514. [PubMed: 15348266]
29. Zheng L, Nakajima M, Higashi T, Foxton RM, Tagami J. Hardness and Young's modulus of transparent dentin associated with aging and carious disease. *Dent Mater J*. 2005; 24:648–653. [PubMed: 16445030]
30. Senawongse P, Otsuki M, Tagami J, Mjör I. Age-related changes in hardness and modulus of elasticity of dentine. *Arch Oral Biol*. 2006; 51:457–463. [PubMed: 16426564]
31. Balooch M, Wu-Magidi I, Balazs A, Lundkvist A, Marshall S, Marshall GW, et al. Viscoelastic properties of demineralized human dentin measured in water with atomic force microscope (AFM)-based indentation. *J Biomed Mater Res*. 1998; 40:539–544. [PubMed: 9599029]
32. Ryou H, Romberg E, Pashley DH, Tay FR, Arola DD. Nanoscopic dynamic mechanical properties of intertubular and peritubular dentin. *J Mech Behav Biomed Mater*. 2012; 7:3–16. [PubMed: 22340680]
33. Habelitz S, Marshall GW, Balooch M, Marshall SJ. Nanoindentation and storage of teeth. *J Biomech*. 2002; 35:995–998. [PubMed: 12052404]
34. Odegard G, Gates T, Herring H. Characterization of viscoelastic properties of polymeric materials through nanoindentation. *Exp Mech*. 2005; 45:130–136.
35. Oliver WC, Pharr GM. An improved technique for determining hardness and elastic modulus using load and displacement sensing indentation experiments. *J Mater Res*. 1992; 7:1564–1583.
36. Hebert EG, Oliver WC, Pharr GM. Nanoindentation and the dynamic characterization of viscoelastic solids. *J Phys D: Appl Phys*. 2008; 41(7):074021.
37. Menard, KP. *Dynamic mechanical analysis: a practical introduction*. 2. Boca Raton: CRC press; 2008.

38. Ryou H, Pashley DH, Tay FR, Arola DD. A characterization of the mechanical behavior of resin-infiltrated dentin using nanoscopic Dynamic Mechanical Analysis. *Dent Mater.* 2013; 29:719–728. [PubMed: 23639453]
39. Jameson M, Hood J, Tidmarsh B. The effects of dehydration and rehydration on some mechanical properties of human dentine. *J Biomech.* 1993; 26:1055–1065. [PubMed: 8408088]
40. Kahler B, Swain MV, Moule A. Fracture-toughening mechanisms responsible for differences in work to fracture of hydrated and dehydrated dentine. *J Biomech.* 2003; 36:229–237. [PubMed: 12547360]
41. Kruzic JJ, Nalla RK, Kinney JH, Ritchie R. Crack blunting, crack bridging and resistance-curve fracture mechanics in dentin: effect of hydration. *Biomaterials.* 2003; 24:5209–5221. [PubMed: 14568438]
42. Balooch M, Habelitz S, Kinney JH, Marshall SJ, Marshall GW. Mechanical properties of mineralized collagen fibrils as influenced by demineralization. *J Struct Biol.* 2008; 162:404–410. [PubMed: 18467127]
43. Ryou H, Niu LN, Dai L, Pucci C, Arola DD, Pashley D, et al. Effect of biomimetic remineralization on the dynamic nanomechanical properties of dentin hybrid layers. *J Dent Res.* 2011; 90:1122–1128. [PubMed: 21730254]
44. Pashley DH, Tay FR, Carvalho RM, Rueggeberg FA, Agee KA, Carrilho M, et al. From dry bonding to water-wet bonding to ethanol-wet bonding. A review of the interactions between dentin matrix and solvated resins using a macromodel of the hybrid layer. *Am J Dent.* 2007; 20:7–21. [PubMed: 17380802]
45. Mutluay MM, Zhang K, Ryou H, Yahyazadehfard M, Majd H, Xu HH, et al. On the fatigue behavior of resin–dentin bonds after degradation by biofilm. *J Mech Behav Biomed Mater.* 2013; 18:219–231. [PubMed: 23276517]
46. Yahyazadehfard M, Mutluay MM, Majd H, Ryou H, Arola DD. Fatigue of the resin–enamel bonded interface and the mechanisms of failure. *J Mech Behav Biomed Mater.* 2013; 21:121–132. [PubMed: 23571321]
47. Angker L, Swain M. Nanoindentation: application to dental hard tissue investigations. *J Mater Res.* 2006; 21:1893–1905.
48. Oyen ML. Nanoindentation hardness of mineralized tissues. *J Biomech.* 2006; 39:2699–2702. [PubMed: 16253265]
49. Lewis G, Nyman JS. The use of nanoindentation for characterizing the properties of mineralized hard tissues: State-of-the art review. *J Biomed Mater Res B Appl Biomater.* 2008; 87:286–301. [PubMed: 18395829]
50. Po J, Kieser J, Gallo L, Tésenyi A, Herbison P, Farella M. Timefrequency analysis of chewing activity in the natural environment. *J Dent Res.* 2011; 90:1206–1210. [PubMed: 21810620]
51. Nalla R, Imbeni V, Kinney JH, Staninec M, Marshall SJ, Ritchie R. In vitro fatigue behavior of human dentin with implications for life prediction. *J Biomed Mater Res A.* 2003; 66:10–20. [PubMed: 12833426]
52. Kruzic JJ, Nalla RK, Kinney JH, Ritchie RO. Mechanistic aspects of in vitro fatigue-crack growth in dentin. *Biomaterials.* 2005; 26:1195–1204. [PubMed: 15451639]
53. Fratzl P, Gupta H, Paschalis E, Roschger P. Structure and mechanical quality of the collagen–mineral nano-composite in bone. *J Mater Chem.* 2004; 14:2115–2123.
54. Zioupos P, Currey J. Changes in the stiffness, strength, and toughness of human cortical bone with age. *Bone.* 1998; 22:57–66. [PubMed: 9437514]
55. Nalla R, Kruzic J, Kinney JH, Balooch M, Ager J III, Ritchie R. Role of microstructure in the aging-related deterioration of the toughness of human cortical bone. *Mater Sci Eng, C.* 2006; 26:1251–1260.
56. Cummings SR, Browner W, Cummings S, Black D, Nevitt M, Browner W, et al. Bone density at various sites for prediction of hip fractures. *The Lancet.* 1993; 341:72–75.
57. Kröger H, Huopio J, Honkanen R, Tuppurainen M, Puntila E, Alhava E, et al. Prediction of fracture risk using axial bone mineral density in a perimenopausal population: a prospective study. *J Bone Miner Res.* 1995; 10:302–306. [PubMed: 7754811]

58. Marshall D, Johnell O, Wedel H. Meta-analysis of how well measures of bone mineral density predict occurrence of osteoporotic fractures. *BMJ*. 1996; 312:1254–1259. [PubMed: 8634613]
59. Bailey A, Sims T, Ebbesen E, Mansell J, Thomsen JS, Mosekilde L. Age-related changes in the biochemical properties of human cancellous bone collagen: relationship to bone strength. *Calcif Tissue Int*. 1999; 65:203–210. [PubMed: 10441651]
60. McCalden R, McGeough J, Barker M, Court-Brown C. Age-related changes in the tensile properties of cortical bone. The relative importance of changes in porosity, mineralization, and microstructure. *J Bone Joint Surg*. 1993; 75:1193–1205. [PubMed: 8354678]
61. Bailey AJ. Molecular mechanisms of ageing in connective tissues. *Mech Ageing Dev*. 2001; 122:735–755. [PubMed: 11322995]
62. Zimmermann EA, Schaible E, Bale H, Barth HD, Tang SY, Reichert P, et al. Age-related changes in the plasticity and toughness of human cortical bone at multiple length scales. *Proc Natl Acad Sci U S A*. 2011; 108:14416–14421. [PubMed: 21873221]
63. Murray PE, Stanley HR, Matthews JB, Sloan AJ, Smith AJ. Age-related odontometric changes of human teeth. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2002; 93:474–482. [PubMed: 12029288]
64. Kruzic J, Ritchie R. Fatigue of mineralized tissues: cortical bone and dentin. *J Mech Behav Biomed Mater*. 2008; 1:3–17. [PubMed: 19627767]
65. Arola DD, Bajaj D, Ivancik J, Majd H, Zhang D. Fatigue of biomaterials: hard tissues. *Int J Fatigue*. 2010; 32:1400–1412. [PubMed: 20563239]
66. Currey JD, Foreman J, Laketi I, Mitchell J, Pegg DE, Reilly GC. Effects of ionizing radiation on the mechanical properties of human bone. *J Orthop Res*. 1997; 15:111–117. [PubMed: 9066534]
67. Currey JD. Role of collagen and other organics in the mechanical properties of bone. *Osteoporos Int*. 2003; 14:S29–S36. [PubMed: 14504703]
68. Zioupos P, Currey J, Hamer A. The role of collagen in the declining mechanical properties of aging human cortical bone. *J Biomed Mater Res*. 1999; 45:108–116. [PubMed: 10397964]
69. Vashishth D, Gibson G, Khoury J, Schaffler M, Kimura J, Fyhrie D. Influence of nonenzymatic glycation on biomechanical properties of cortical bone. *Bone*. 2001; 28:195–201. [PubMed: 11182378]
70. Wang X, Shen X, Li X, Mauli Agrawal C. Age-related changes in the collagen network and toughness of bone. *Bone*. 2002; 31:1–7. [PubMed: 12110404]
71. Miura J, Nishikawa K, Kubo M, Fukushima S, Hashimoto M, Takeshige F, et al. Accumulation of advanced glycation end-products in human dentine. *Arch Oral Biol*. 2014; 59:119–124. [PubMed: 24370182]
72. Launey ME, Buehler MJ, Ritchie RO. On the mechanistic origins of toughness in bone. *Annu Rev Mater Res*. 2010; 40:25–53.
73. Kinney JH, Pople J, Marshall GW, Marshall SJ. Collagen orientation and crystallite size in human dentin: a small angle X-ray scattering study. *Calcif Tissue Int*. 2001; 69:31–37. [PubMed: 11685431]
74. Deymier-Black AC, Yuan F, Singhal A, Almer JD, Brinson LC, Dunand DC. Evolution of load transfer between hydroxyapatite and collagen during creep deformation of bone. *Acta Biomater*. 2012; 8:253–261. [PubMed: 21878399]
75. Kim YK, Mai S, Mazzoni A, Liu Y, Tezvergil-Mutluy A, Takahashi K, et al. Biomimetic remineralization as a progressive dehydration mechanism of collagen matrices –implications in the aging of resin-dentin bonds. *Acta Biomater*. 2010; 6:3729–3739. [PubMed: 20304110]
76. Smith A, Denny M. High-frequency oscillations as indicators of neural control mechanisms in human respiration, mastication, and speech. *J Neurophysiol*. 1990; 63:745–58. [PubMed: 2341873]
77. Xia Y, Bigerelle M, Marteau J, Mazeran P-J, Bouvier S, Iost A. Effect of surface roughness in the determination of the mechanical properties of material using nanoindentation test. *Scanning*. 2014; 36:134–149. [PubMed: 23835928]
78. Kim J-Y, Lee J-J, Lee Y-H, Jang J-I, Kwon D. Surface roughness effect in instrumented indentation: A simple contact depth model and its verification. *Journal of Materials Research*. 2006; 21:2975–2978.

Figure 1.

Micrographs of old and young dentin obtained by FE-SEM. a) Old dentin at x5000 magnification, b) a single tubule of old dentin at x25000 magnification, c) young dentin at x5000 magnification, d) a single tubule of young dentin at x25000 magnification. Note that the high magnification images in (b) and (d) are from the highlighted regions in (a) and (b), respectively.

Figure 2.

Results from nanoDMA of old dentin. a) complex, b) loss, c) storage and d) $\tan\delta$ of the intertubular and peritubular dentin. Upper case letters indicate there are significant differences. Note that there is a significant difference ($p < 0.05$) between the elastic responses of the intertubular and peritubular components of old dentin as evident from the storage modulus distribution shown in (c). There is no significant difference between the viscoelastic responses of the intertubular dentin and peritubular components as evident from the similarities of the loss modulus and $\tan\delta$ in (b) and (d), respectively.

Figure 3.

A comparison of the nanoDMA responses for young and old intertubular dentin. a) complex, b) loss, c) storage and d) $\tan\delta$. Upper and lower case letters indicate significant differences in the old and young dentin, respectively, with respect to loading frequency. Note that the elastic responses evident from the storage modulus in (c) do not show frequency dependency ($p > 0.05$) for either age group, whereas the viscoelastic responses in (b) and (d) do exhibit frequency dependency ($p < 0.05$). The complex and storage moduli of old dentin were not significantly different ($p > 0.05$) than those for young dentin, regardless of frequency. Above 10 Hz frequency, the dampening components for old dentin (E'' and $\tan\delta$) are significantly less than that for young dentin as evident in b and d, except at 100 Hz.

Figure 4.

A comparison of the nanoDMA responses for young and old peritubular dentin. a) complex, b) loss, c) storage and d) $\tan\delta$. Upper and lower case letters indicate a significant difference in the old and young dentin, respectively, with respect to loading frequency. Note that the elastic responses evident from the storage modulus in (c) do not show frequency dependence ($p > 0.05$), whereas the viscoelastic responses in (b) and (d) do exhibit frequency dependence ($p < 0.05$). The complex and storage moduli of old dentin were not significantly different ($p > 0.05$) from those of young dentin, regardless of frequency. There was a significant difference in the dampening behavior between the two age groups. Above 20 Hz frequency, the dampening components for old dentin (E'' and $\tan\delta$) are significantly less than that for young dentin as evident in b and d, except at 100 Hz.

Figure 5.

Property maps for an old dentin specimen obtained using nanoDMA in scanning mode. a) complex modulus [GPa] ($E^* = 19.6 \pm 10.1$ GPa), b) loss modulus [GPa] ($E'' = 5.7 \pm 6.2$ GPa), c) storage modulus [GPa] ($E' = 16.3 \pm 7.5$ GPa), d) topography [nm] and e) $\tan \delta$ (0.47 ± 0.59). Note the larger elastic/storage modulus of the peritubular dentin ($E^* = 34.8 \pm 17.5$ GPa, $E' = 26.1 \pm 14.4$ GPa) in comparison to the intertubular dentin ($E^* = 17.7 \pm 6.6$ GPa, $E' = 14.8 \pm 5.5$ GPa).

Figure 6.

The complex modulus distribution within individual tubules of old dentin completely filled with mineral. a) Definition of the dimensions used for normalizing radial distance for the peritubular cuffs. The radius of the peritubular cuff (R^*) is measured for each of the tubules evaluated and the complex modulus was obtained with respect to the distance from the center (r = actual radial distance $0 < r \leq R^*$). Measurements were taken for four cuffs of each sample and averaged. b) Complex modulus distribution as a function of radial distance. The complex modulus of the deposited mineral with aging near the center of the tubule (approximately 22 GPa) is significantly lower than that of the original peritubular cuff (approximately 35 GPa).

Figure 7.

Micrographs of single tubules from the dentin of an old donor tooth using FE-SEM. a) secondary electron image, and b) the corresponding backscattered electron image. Note the lower grayscale apparent within the region close to the center of the lumen in the backscattered image. A high magnification view of a partially occluded tubule lumen in an old dentin specimen. Note the larger crystal size within the lumen in relation to those evident with the original peritubular cuff. The effective size of crystals inside the lumen is approximately 100 nm, which is in agreement with the dimensions reported in Porter et al., [14].