

Available online at www.sciencedirect.com**ScienceDirect**journal homepage: www.intl.elsevierhealth.com/journals/dema

Root fractures in seniors: Consequences of acute embrittlement of dentin



W. Yan^a, H. Chen^b, J. Fernandez-Arteaga^c, A. Paranjpe^d, H. Zhang^e,
D. Arola^{a,e,f,*}

^a Department of Materials Science and Engineering, University of Washington, Seattle, WA, USA

^b Department of Periodontics, School of Dentistry, University of Washington Seattle, WA USA

^c School of Engineering, Universidad EAFIT, Medellín, Colombia

^d Department of Endodontics, School of Dentistry, University of Washington Seattle, WA USA

^e Department of Restorative Dentistry, School of Dentistry, University of Washington Seattle, WA USA

^f Department of Oral Health Science, School of Dentistry, University of Washington Seattle, WA USA

ARTICLE INFO

Article history:

Received 24 August 2020

Accepted 29 August 2020

Keywords:

Aging

Dentin

Root fracture

Dynamic mechanical analysis

ABSTRACT

Dentin undergoes irreversible changes in microstructure with aging that involve gradual filling of the tubule lumens with mineral. Known as dental sclerosis, this process begins at the root apex, progresses coronally, and is associated with a degradation in the resistance to fracture of dentin.

Objective. To determine i) age-related changes of intertubular dentin with aging, particularly within the root, and ii) the differences in age-related degradation between vital and pulpless (i.e. non-vital) teeth.

Methods. We performed nanoscopic dynamic mechanical analysis (nanoDMA) in scanning mode on the intertubular and peritubular dentin of teeth from young and old adults. The complex, loss and storage moduli, as well as the tan delta parameter were evaluated for teeth with no restorations and teeth with root canal treatment (non-vital).

Results. There were significant changes in the dynamic moduli of intertubular dentin with age, which were most substantial in the apical third of the root. The storage modulus of the intertubular dentin, which quantifies the purely elastic resistance to deformation, was significantly ($p < 0.0005$) larger for both the old vital and non-vital teeth than that of the young teeth, over the entire root length. However, the tan delta parameter, which quantifies the relative capacity for viscous deformation, was significantly lower in these two groups ($p < 0.005$).

Significance. Radicular dentin undergoes an embrittlement with aging, involving reduced capacity for viscous deformation. The extent of degradation is largest in the apical third. Removal of the pulp appears to accelerate the aging process or compound the extent of degradation.

© 2020 The Academy of Dental Materials. Published by Elsevier Inc. All rights reserved.

* Corresponding author at: Department of Materials and Engineering, University of Washington, Roberts Hall, 333, Box 352120, Seattle, WA, USA.

E-mail address: darola@u.washington.edu (D. Arola).

<https://doi.org/10.1016/j.dental.2020.08.008>

0109-5641/© 2020 The Academy of Dental Materials. Published by Elsevier Inc. All rights reserved.

1. Introduction

An increase in life expectancy coupled with rapid advancements in oral healthcare have led to an increase in the population of partially and fully dentate seniors over past decades [1]. As a consequence, there will be growth in the population of seniors seeking dental care. The increase in dentate seniors has brought new challenges to the profession. One such concern is cracked teeth and other forms of mechanical degradation [2,3]. While tooth fractures are routinely encountered in dental practice irrespective of patient age [4], vertical root fractures most commonly occur in patients 40 to 60 years old [5]. These types of failures cannot often be repaired successfully by root canal treatment (RCT) and necessitate tooth extraction [6].

Tooth fractures that initiate within the root, or that extend from the line angles within the crown typically involve dentin. Dentin is highly mineralized tissue consisting of approximately 45 % mineral by volume [7] and serves as a foundation for the enamel. It is traversed by a network of microscopic channels (i.e. tubules) that extend radially from the pulp towards the dentin-enamel junction (DEJ) and cementum. The tubule lumens are occupied by dentin fluid and odontoblastic extensions. They are bounded by a highly mineralized cuff of peritubular dentin that consists primarily of apatite crystals. The region between the tubules is occupied by the intertubular dentin, which consists of a mesh of collagen fibrils that is reinforced by inter- and extra-fibrillar apatite crystallites.

The microstructure of dentin in adults is dynamic. It undergoes a gradual transition in translucency with increasing age that results from filling of the dentin tubules with mineral, a process regarded as sclerosis [8]. Dentin sclerosis occurs first and is most severe in tissue near the root apex [9], which could be attributed to the tubule morphology in the root. The dentin tubules have a smaller lumen diameter and density in the root in relation to the crown [2].

Although the mechanisms of dentin sclerosis are unclear [10,11], the consequences are far more well known. In particular, the age-related changes in microstructure of dentin reduce the damage tolerance of the tooth [12]. Previous studies that addressed aging and the mechanical behavior of dentin have shown that the crown [13] and root [14] undergo reductions in strength. Furthermore, the fatigue strength [15,16], fracture toughness [17,18] and fatigue crack growth resistance [19,20] also undergo a significant reduction with age. However, there are two key limitations of prior studies on this topic, namely: i) most work has concentrated on the coronal dentin, and ii) investigations of the root have not considered spatial variations. Furthermore, the degradation in damage tolerance of dentin with aging has largely been attributed to what is most noticeable, i.e. the mineral that fills the dentin tubules. In comparison, far less effort has focused on the changes to the intertubular dentin.

Natural physiological aging is not the only concern in root fractures. Teeth with non-vital pulp and those that have had the pulp removed during root canal treatment (RCT) are more susceptible to fracture than vital teeth [21]. Vertical root fracture (VRF) occurs more frequently in teeth with prior RCT [22]. According to the American Association of Endodontists,

a VRF involves cracks that initiate in the apical region [23] and extend coronally (Fig. 1A). There is some belief that VRFs initiate from internal dentin cracks [24] that have resulted from the RCT. Indeed, dentin defects introduced during the endodontic procedures could be a contributing factor [25–27]. However, previous studies have also been reported that no defects could be identified within the dentin of teeth subjected to RCT and/or their contribution to VRF is unlikely [28,29]. Clearly the cause of VRF and how RCT contributes to the process of failure is controversial and remains unclear [30].

Removal of the pulp during RCT is associated with irreversible change to the dentin structure [31]. In a comparison of the strength of root dentin between teeth with age- and donor-matched controls, those with prior RCT exhibited 30% lower strength [32]. Yet, the progression of aging in non-vital teeth, specifically those that have received RCT followed by post-treatment function, and the spatial variations in degradation of the root are not understood. Root fractures in the teeth of seniors with RCT could be attributed to an acute form of degradation in mechanical properties at the apex and could manifest in the intertubular or peritubular components of dentin. Understanding this phenomenon is the first step towards the development of treatment modalities, or the development of dental materials, that account for the unique property variations.

Indentation methods have been adopted to characterize the changes in mechanical behavior of dentin with aging and sclerosis [33,34]. Macroscopic indentation methods are not able to discern the properties of distinct aspects of the microstructure or interfaces [35], which is highly relevant to an evaluation of dentin. As such, nanoindentation has become a standard method for measuring the mechanical behavior of biological hard tissues at the microscopic scale and also enables an assessment of the viscous behavior. Nanoindentation or atomic-force microscopy is required to measure discrete changes in the intertubular and peritubular dentin [36], which is relevant to the present investigation.

Nanoscoptic Dynamic Mechanical Analysis (NanoDMA) is a special form of nanoindentation-based structural analysis. Scanning nanoDMA, performed using scanning probe microscopy, maintains the indentation stress within the elastic range, which is preferred for highly sensitive analyses. Recent applications include evaluations of dentin bonding [37] and aging [38]. It has also been applied in investigations related to remineralization of dentin [39–42] and to assess the effects of collagen crosslinking [41,43]. However, no investigation has been reported on the use of nanoDMA mapping within tooth roots to delineate the unique age-related changes of the intertubular and peritubular dentin or the differences between vital and non-vital teeth.

In the present study, scanning mode nanoDMA was used to characterize the dynamic mechanical behavior of dentin in the roots of teeth with regards to donor age, pulp vitality and histological location. Two null hypotheses were defined: i) there is no significant difference in the mechanical behavior of intertubular dentin along the length of the root with regards to donor age, and ii) there is no significant difference in the properties of intertubular dentin between vital teeth and those with prior RCT.

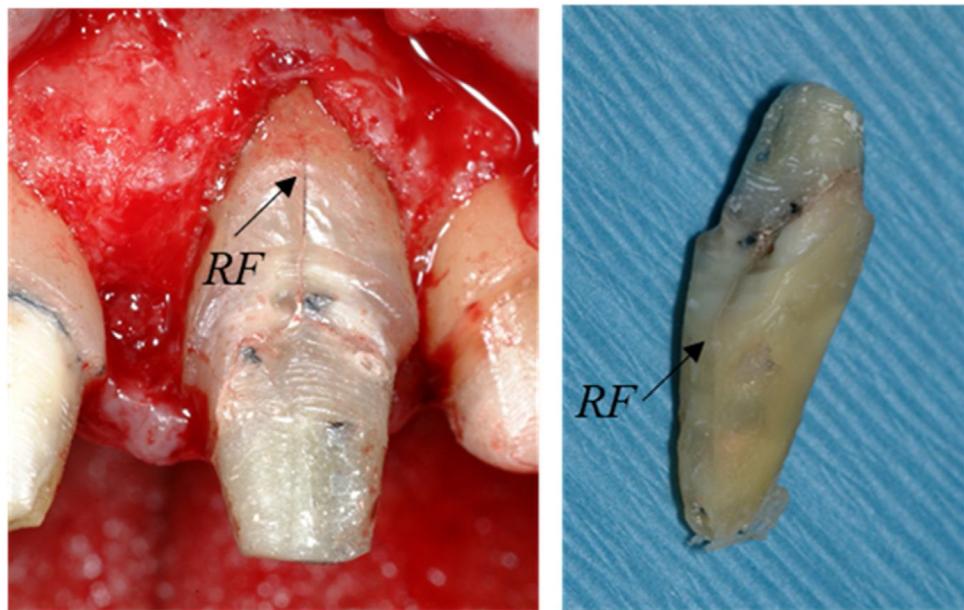


Fig. 1 – Root fracture originating from the apex in tooth #9 of a 69-year-old male patient. This tooth is not included in the samples evaluated.

2. Materials and methods

Human single-rooted non-carious teeth were obtained from participating clinics with an exempt protocol approved by the Institutional Review Board of the University of Washington. Single-rooted premolars were chosen for their simple physiology in comparison to molars with multiple roots. The teeth were stored in Hank's balanced salt solution (HBSS) with record of donor age and gender. Those teeth with visible caries or structural defects were discarded. A total of 12 teeth from 8 patients were selected and divided into young ($n = 4$, age <25), old ($n = 4$, age >60) and old non-vital ($n = 4$, age >60) groups. The four teeth in the old and old non-vital groups were matched pairs of teeth. Each pair consisted of teeth from mirrored locations of the arch from the same donor that included a vital tooth and non-vital tooth with prior RCT. Obtaining matched pairs of teeth in mirrored locations of the arch is very difficult, but very beneficial, since it reduces the variations in tooth properties across donors.

The teeth were cast in a polyester resin foundation within two weeks of receipt and sectioned axially in the mesial-distal (M-D) direction using a precision slicing/grinding machine according to established methods [14]. The resulting halves were embedded in cold-cured epoxy resin (Epoxy HQ Resin and Hardener, Struers) exposing the root canal and longitudinal section. The exposed dentin sections were polished using silicon carbide abrasive paper from #800 to #4000 mesh with water irrigation until halfway through the thickness of dentin and the dentin tubules became evident. Further polishing was performed with 3 μm diamond particle suspensions and 0.04 μm colloidal alumina suspension. The specimens were subjected to 20 minutes sonication to remove residual debris inside the dentin tubules. All of the aforementioned procedures were conducted with the tooth maintained fully hydrated in HBSS.

A dynamic mechanical analysis (DMA) of the polished sections of root dentin was performed using Scanning Probe Microscopy (SPM) on a commercial system for nanoindentation (Hysitron Inc., Model TI980 Triboindenter, Minneapolis, MN). The scanning-based evaluations were performed using a Berkovich diamond indenter with 90 nm nominal tip radius, which was measured in scanning mode with quartz standard according to [37]. Prior to nanoDMA on teeth, a frequency sweep was performed on fused quartz from 100 Hz to 300 Hz to identify resonance components related to the machine operation. Based on the results of this process a scanning frequency of 200 Hz was used to maximize the signal to noise ratio in the evaluations of dentin. A 4 μN static indentation load and a dynamic sinusoidal load of 2 μN were applied following Ryou et al. [44] The scanning mode nanoDMA was conducted using a window of evaluation of 20 $\mu\text{m} \times 20 \mu\text{m}$, which constitutes an area involving 5–10 dentin tubules as shown in Fig. 2A. The instrument performs the evaluation through 256 horizontal scans and with pixel density of 256 \times 256. Properties of the intertubular and peritubular dentin were quantitatively evaluated within the apical, middle and coronal thirds (Fig. 2B). In each region, areas of interest within the window of evaluation were selected that corresponded to either the intertubular or peritubular dentin. Three independent nanoDMA scans were conducted in each region of each tooth to ensure that a statistical representative assessment was achieved. Then the average and standard deviation of the desired properties were determined from the scan data within that domain of relevance.

To minimize the effects of dehydration to the mechanical behavior [45], the nanoDMA evaluations were performed in the hydrated condition with treatment of 99.4% ethylene glycol (EG) [38]. Briefly, a thin layer of EG was smeared on the sample surface after removal from HBSS bath to prevent the evaporation of water during scanning. Previous results have shown that there is no influence of EG on the moduli estimated using

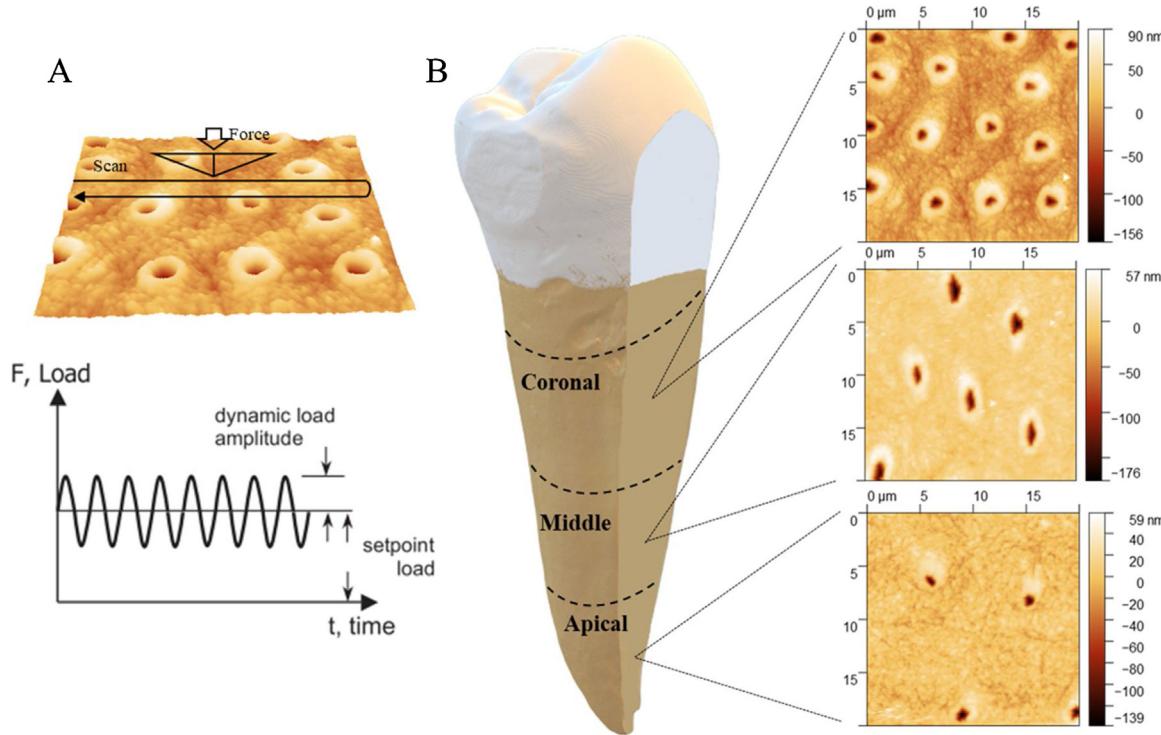


Fig. 2 – Details of the experimental evaluation. A) nanoDMA scanning on prepared dentin surface using scanning probe microscopy (SPM) with a Berkovich indenter. Under load control, the indentation involves a static component (setpoint load) and a superposed dynamic load amplitude equivalent to 50% of the static component. B) Demonstration of analysis within the three chosen locations along the tooth root using SPM. The root is divided into the coronal-, middle- and apical-thirds. Representative topography maps show the lumen density within each of these three regions. Note the progressively lower tubule density from the coronal to the apical third of the root in B.

scanning-based DMA [44] and that this approach can maintain the hydration of the tissue for over an hour. Since the individual scans were completed with a period of less than 30 min, the process ensured that the properties were evaluated in the fully hydrated condition.

Within each region of evaluation, the storage (E') and loss (E'') moduli were obtained from the nanoDMA scans, which 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. 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 independent of tip geometry. A statistical analysis of the parameters was performed using a two-way Analysis of Variance (ANOVA) test with significant differences identified at $\alpha = 0.05$. The normality of the data was checked before performing the statistical analysis.

3. Results

Representative property maps obtained for the apical dentin of a tooth root from the young group of donors are shown in Fig. 3. Specifically, the surface topography and mechanical properties distributions representing the complex, storage and loss

moduli, as well as the tan delta distribution are presented in this figure as denoted. These maps were obtained over a $20 \mu\text{m} \times 20 \mu\text{m}$ window of evaluation. The dentin tubules and peritubular cuffs are clearly evident in the topography and mechanical property maps, which enabled precise identification of the regions corresponding to the intertubular and peritubular dentin. The center of the tubule lumens exhibit property values that are unrealistic due to boundary effects between the tip and open lumens. Such areas are artifacts of the indentation method and were excluded in the quantitative analysis.

Representative property maps for the apical dentin of roots within the vital and non-vital old groups are presented in Fig. 4(a) and (b), respectively. Shown are the property distributions for the storage and loss moduli, as well as the tan delta parameter. Note that the maps for the individual properties presented in Figs. 3 and 4 have the equivalent range for easy comparison. Differences in the complex and storage modulus between the young and old dentin are clearly evident. Specifically, the storage modulus measurements for the intertubular dentin of both old groups are substantially higher than those of the young teeth. In contrast, the tan delta values for the intertubular dentin of the old groups appear lower than those obtained for the young dentin.

A comparison of the dynamic mechanical properties of the peritubular dentin from the apical-, middle- and coronal-thirds of the roots was performed and results are shown in

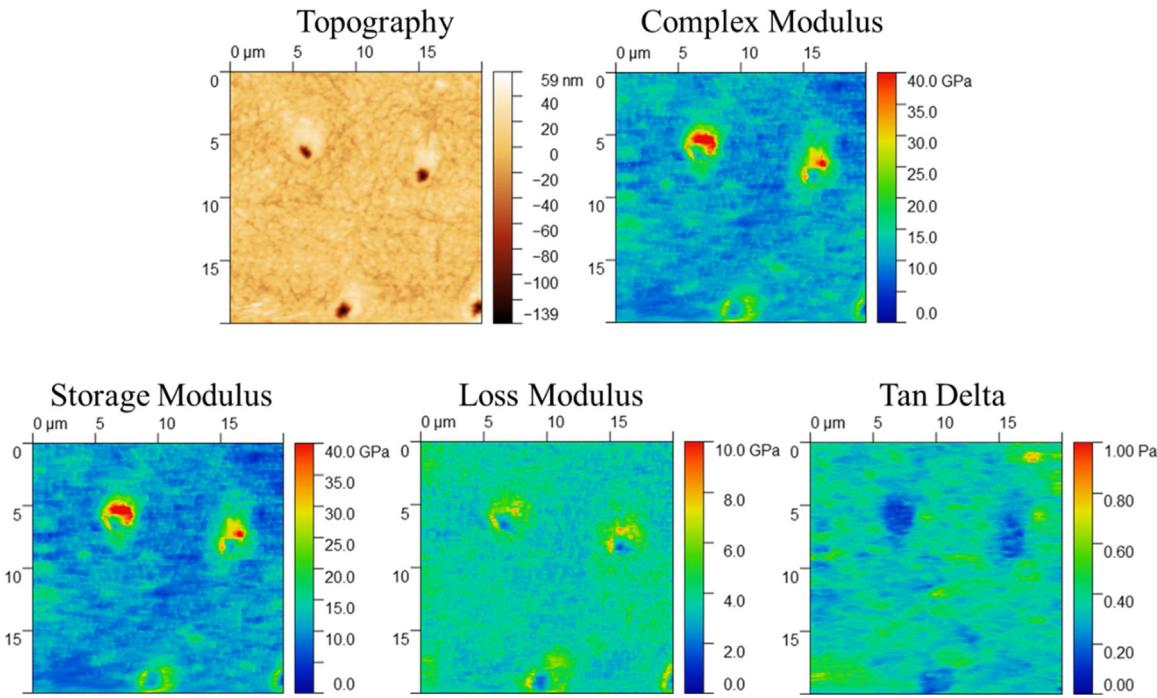


Fig. 3 – Representative property maps from the apical-third of a tooth that is part of the young vital group. Note that the maps include the surface topography, complex modulus, storage modulus, loss modulus and tan delta as labeled. The scan area involves three mostly complete dentin tubules, and partial view of a fourth, which are clearly evident in the scans.

Fig. 5. Specifically, the storage and loss moduli are presented in Fig. 5A and B, respectively, and the tan delta parameter and complex modulus are shown in Fig. 5C and D, respectively. In all three locations of the root, the dynamic mechanical properties of the peritubular dentin from the old vital and non-vital teeth were not significantly different ($p > 0.05$) from those of the young teeth. However, with regards to spatial variations, the storage and complex moduli of the peritubular dentin from the old vital roots in the apical third were significantly greater ($p < 0.05$) than those values for the measures from middle- and coronal-thirds. Furthermore, there was no significant difference in the measured tan delta parameter of the peritubular dentin between the age groups and with respect to location.

Similar to the analysis of the peritubular dentin in Fig. 5, quantitative comparisons of the dynamic mechanical properties of the intertubular dentin are presented in Fig. 6. Specifically, the storage and loss moduli for tissue from the young, old and old non-vital roots are compared in Fig. 6A and B, respectively. Similar comparisons of the tan delta and complex modulus of the intertubular dentin are shown in Fig. 6C and D, respectively. Regarding spatial variations, the storage and complex moduli of the intertubular dentin obtained from the old vital teeth were significantly larger ($p < 0.05$) in the apical third than in the two regions of interest located more coronally. That warrants rejection of the first null-hypothesis. In contrast, no spatial variations were identified in the properties of the young or the old non-vital teeth over the root length. Regarding differences between age groups, the intertubular dentin of the old vital teeth exhibited significantly higher ($p < 0.0005$) storage and complex moduli in the apical- and middle-thirds than the young dentin. Furthermore, the

complex moduli of the intertubular dentin from roots of the old non-vital group were significantly higher in all three locations ($p < 0.0005$). That difference requires rejection of the second null hypothesis. While there was no difference in the loss modulus across age groups, the tan delta parameters of the young dentin were significantly higher than the two old groups, regardless of location ($p < 0.005$).

4. Discussion

According to an investigation using discrete indent mode nanoDMA, Ryou et al. [38] reported that there was no significant difference in the storage and complex moduli of peritubular dentin between young and old vital teeth. Those findings were confirmed through results of the present study in scanning mode. Nevertheless, the peritubular dentin of the old teeth in Ryou et al. [38] was reported to have significantly lower loss modulus and tan delta, implying that it has a lower capacity for viscous dampening. That trend was not observed by nanoDMA in scanning mode. The difference could be attributed to the mechanics of indentations and/or size effects associated with application of the two distinct methods. Based on its spatial relation and sensitivity, scanning nanoDMA is a more appropriate method for assessing the properties of the individual constituents. The set point load in the scanning mode used herein was 4 μ N, which is 100 times lower than in the discrete indent mode performed by Ryou et al. [38]. The high sensitivity of the scanning mode analysis enabled isolating properties of the peritubular cuff from effects of its surroundings; no significant differences with age were found. Moreover, the studies by Ryou et al. [37,38] were conducted on

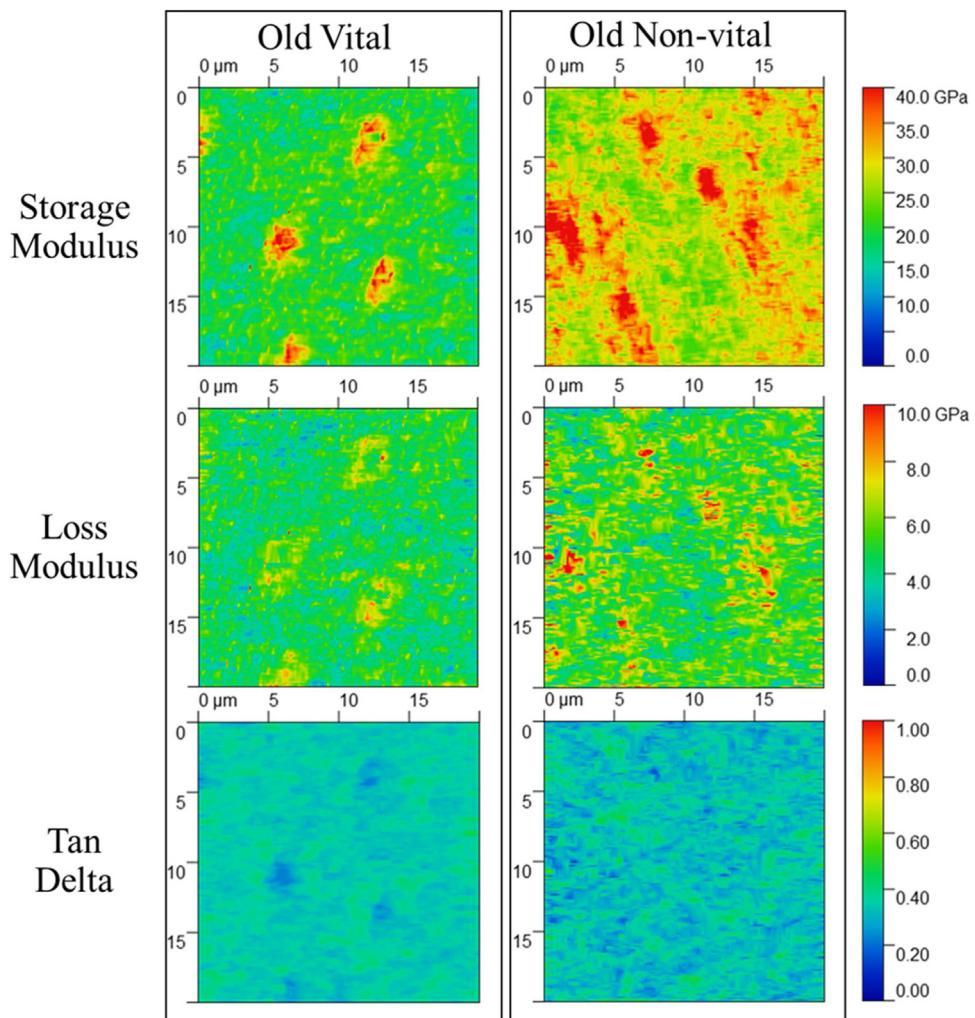


Fig. 4 – Property maps of the dynamic mechanical properties for dentin from the apical thirds of teeth representing the old vital and old non-vital groups as labeled. The nanoDMA maps for these two teeth were obtained from donor-matched tooth pairs.

coronal dentin and not on tissue from the root. It is important to acknowledge the difference in the tissue from these two regions. There are no changes in the dynamic mechanical properties of the peritubular cuffs of radicular dentin with aging, except for within the apical third. Within the two old groups, there was no significant difference in the properties of the peritubular cuff between the radicular dentin of vital and RCT non-vital teeth.

To the authors' knowledge, no previous study has evaluated the spatial variation in properties of the peritubular dentin in the root. As sclerosis starts from root apex in the third decade of life [46], it was expected that the apical-third in the old groups, where the sclerosis is most advanced, could have higher storage and complex moduli. Indeed, there was an increase in these two moduli at the root apex of the two old groups (Fig. 5A and D). Deposition of mineral within the dentin tubules can fundamentally change the indentation response. Occlusion of the dentin tubules changes the physical boundary conditions of the cuffs by suppressing the degree of transverse elastic deformation. Another possible mecha-

nism is densification of the cuffs through a precipitation of crystals stimulated by the aqueous solution within the dentin tubules. The latter explanation is supported by the smaller crystal size in aged dentin with respect to that in the cuffs of young dentin [47]. Nevertheless, it only applies to the vital teeth, not those that have received RCT. The specific cause for the larger resistance to elastic deformation exhibited by the peritubular cuffs in the two old groups is unclear.

In comparison to the effort placed on the dentin tubules with aging, the evolution in properties of intertubular dentin has received limited attention. In bone, the age-induced degradation in toughness is almost exclusively attributed to the collagen matrix [48]. In teeth, intertubular dentin occupies more than 90% of the volume of dentin overall and type-I collagen accounts for approximately 90% of the intertubular matrix [7]. As such, volumetrically, the intertubular dentin is more critical to resisting tooth fracture and the collagen fibril mesh is integral to its fracture toughness [49]. By virtue of its large volume fraction, property alterations of the collagen and intertubular dentin with aging are potentially more detri-

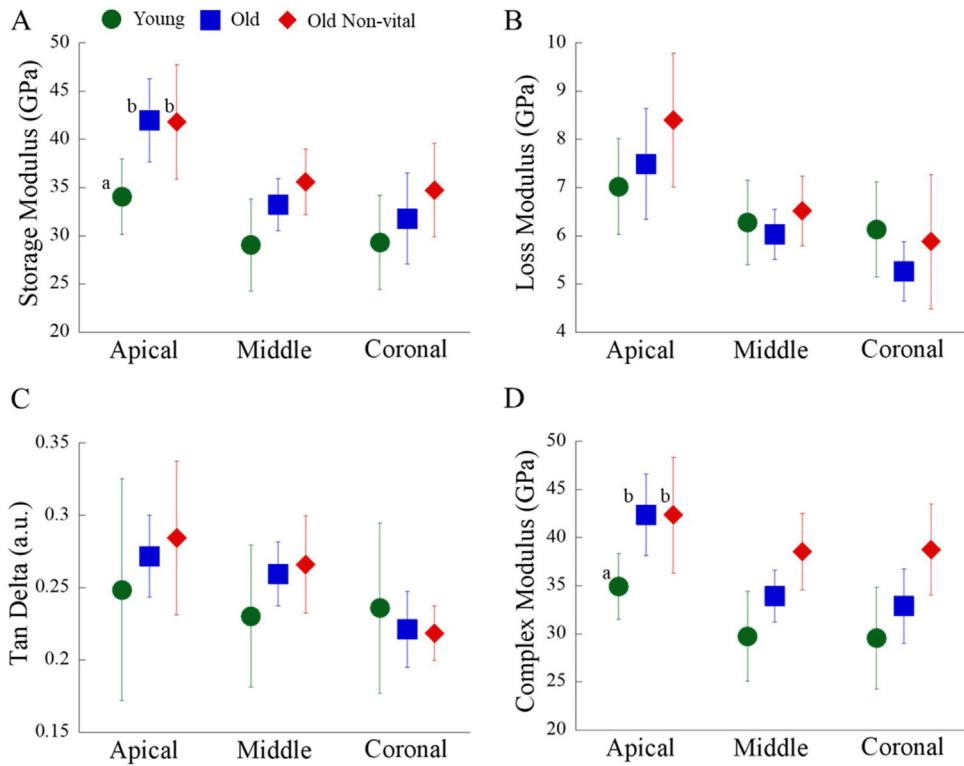


Fig. 5 – A comparison of the nanoDMA responses for young, old and old non-vital peritubular dentin. Data points within each region labeled with different letter are significantly different ($p < 0.05$). A) Storage Modulus, B) Loss Modulus, C) Tan Delta, D) Complex Modulus.

mental overall than the changes to the peritubular cuff and dentin tubules. This statement is most applicable to the apical third of the root where the tubule density is the lowest overall (Fig. 2B). As sclerosis begins in the third decade of life [46], the apical region undergoes sclerosis for much longer time than the crown. Consequently, age-related changes in properties of dentin would be expected to be largest near the apex. Indeed, that is where the most significant changes in properties of the old vital dentin were observed (Fig. 6).

Kinney et al. [15] proposed a dissolution-reprecipitation theory for sclerosis where dissolution of intertubular mineral is reprecipitated within the tubules as magnesium-rich beta-tricalcium phosphate and apatite. And in support of that mechanistic theory, sclerotic dentin exhibits a reduction in crystal size in the intertubular region [10]. Due to the reduction in volume fraction of mineral within the intertubular space, the complex and storage modulus of intertubular dentin would be expected to decrease with age, not increase. Both the old vital and non-vital intertubular dentin exhibited significantly higher storage and complex modulus, regardless of location (Fig. 6). The larger storage modulus could be caused by an increase in the mineral content or perhaps a greater extent of collagen cross-linking. Yan et al. [14] recently showed that there are increases in both the mineral to collagen ratio and the extent of cross-linking in the root with aging. Although that study was limited to vital teeth, a later study involved the roots of RCT teeth from old donors [32] and showed that non-vital teeth exhibited lower mineral to collagen ratio than

that of vital teeth, but significantly larger extent of collagen cross-linking. That implies that the larger storage and complex moduli of the vital and non-vital groups could result from different mechanisms. Without more information regarding the specifics of the RCT performed, it is not possible to distinguish the causes to the increase in cross-linking in that study. It appears that more work is required to elucidate the aging process in non-vital dentin.

The increase in storage modulus of the intertubular dentin with age results in detrimental consequences. For the same stress applied to an arbitrary volume of intertubular dentin (Fig. 7A), the young dentin with comparatively low storage modulus (denoted as E for old dentin and E/2 for young dentin), can accommodate much greater strain energy to a critical stress (Fig. 7B). The strain energy that manifests beyond what can be stored is converted to energy for fracture. This is where viscous deformation in dentin plays a very important role. The tan delta quantifies the relative dampening capacity, which involves viscous relaxation to an applied stress as shown schematically in Fig. 7C. With higher loss modulus, young dentin possesses more viscous relaxation (dark arrow), and thus dissipate more strain energy. The nearly 25% decrease of tan delta in old dentin indicates diminished capacity for viscous stress relaxation in relation to young dentin. The loss of this capacity for stress relaxation through viscous behavior is expressed as an embrittlement of the tissue. Therefore, an increase in root fractures in senior patients is quite conceivable due to this embrittlement of the intertubular dentin.

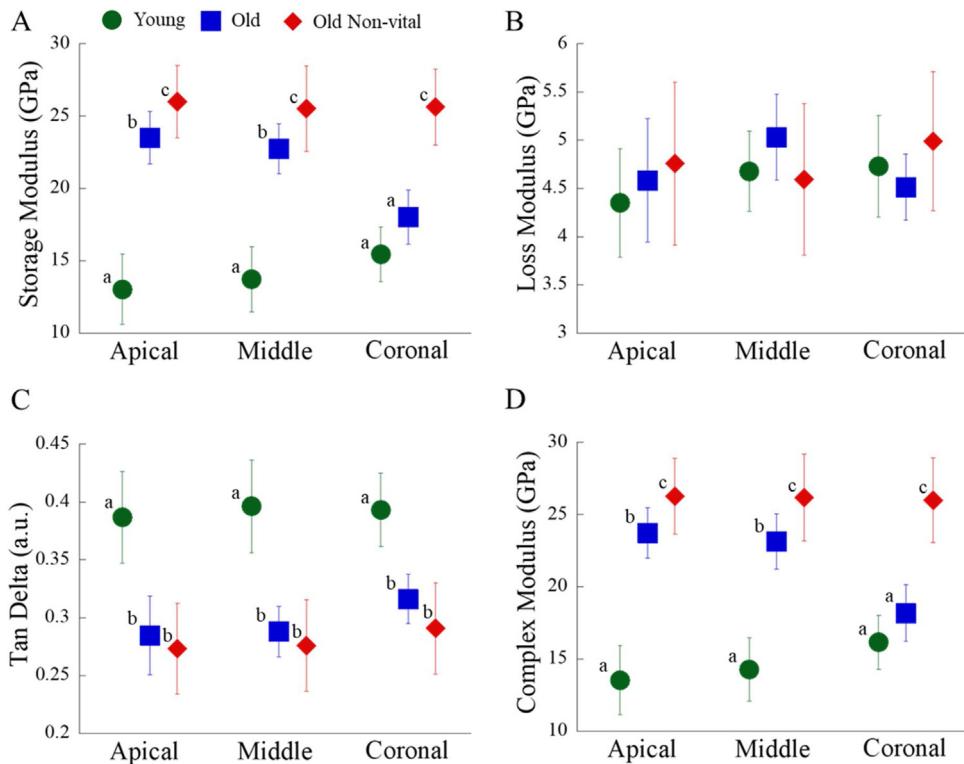


Fig. 6 – A comparison of the nanoDMA responses for young, old and old non-vital intertubular dentin. Data points within each region that are labeled with different letters are significantly different ($p < 0.05$). A) Storage Modulus, B) Loss Modulus, C) Tan Delta, D) Complex Modulus.

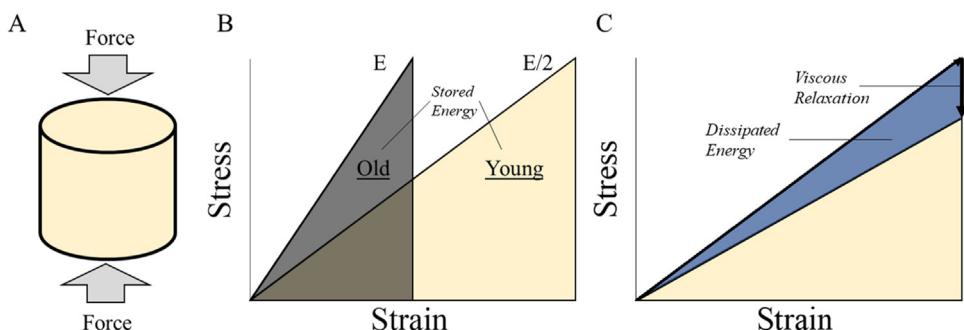


Fig. 7 – Schematic representation of the storage and loss moduli and their importance to the mechanical behavior of dentin. A) A cylindrical volume of dentin subjected to compressive force that results in uniaxial stress and strain. B) A comparison of the strain energy stored to failure for young and old dentin shown by the area under the stress-strain curves (assuming equivalent strength). Notice the lower strain energy storage capacity of the old dentin due to its high elastic (i.e. storage) modulus. C) Viscous relaxation in young dentin (noted in blue) results in a reduction of the axial stress and a decrease in the strain energy within the material generated by mechanical loading. That relaxation reduces the propensity for root fracture due to the reduction in strain energy (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

There is precedence for cross-linking of the collagen to play an important role in the embrittlement with aging, especially in the non-vital teeth (Fig. 6D). In bone, collagen crosslinking contributes to its decline in damage tolerance with age [50,51]. Dentin undergoes accelerated collagen crosslinking [52], which contributes to a reduction in strength with age [15]. A previous study has also shown that there is a correlation between intertubular collagen crosslinking and reduction

of fracture strength in donor-matched RCT and non-RCT root dentin [30]. Thus, crosslinking of the collagen matrix within the intertubular dentin is a potential contribution to the increase in storage and complex moduli overall, as well as to the changes in the apical third. This comment requires further support and assessment of the intertubular mineralization to establish that it is not a confounding factor to the changes in properties.

One of the most interesting finding is the significant differences in properties between vital and non-vital dentin of the donor matched pairs (Fig. 6). Structural changes in dentin after removal of the pulp are unexpected due to absence of cellular processes as well as loss of the dentin fluid and internal pulpal pressure. Consequently, the driving forces causing the evolution of sclerosis are deactivated. However, the property maps clearly showed that non-vital dentin possessed significantly higher storage and complex moduli than the vital counterparts, and along the entire length of the tooth. This is the first report of such changes in properties of RCT teeth. Although the dynamic mechanical properties for the old dentin exhibited gradients from the apical- to coronal-thirds, indicating more advanced aging near the apex, no such spatial variations were evident in the non-vital dentin. It is reasonable to speculate that the collagen in pulpless teeth undergoes crosslinking or denaturation as a consequence of the treatment. For example, sodium hypochlorite irrigation pretreatment of dentin has been proven to effect the elastic modulus and hardness and potentially cause collagen alteration [53,54]. Although plausible, this topic requires further investigation.

There are limitations to the present investigation. One of the most critical concerns is the unknown dental history of the donors. While the microstructure of dentin appears consistent among all teeth, its properties are unique from person to person [32]. This is a consequence of many factors, which may include diet, medications, parafunctional habits, etc. The patient factor was reduced by using donor-paired vital and non-vital teeth. Nevertheless, differences in the progression of aging between the donors is uncertain. Furthermore, no details were available regarding the RCT teeth, including method and time of treatment, or the period of post-treatment function. These are important factors in understanding the initiation and progression of degradation. There was also a limitation in assessing the specific changes in the microstructure and chemical composition responsible for the decrease in capacity for viscous deformation at the sub-micron level. Advanced techniques such as atom probe tomography should be applied to examine the structural changes in dentin with aging at the atomic level.

5. Conclusion

Scanning mode nanoDMA was utilized to evaluate the dynamic mechanical properties of dentin from the roots of adult donor teeth as a function of donor age, pulp vitality and histological location. The intertubular dentin of the old group exhibited higher storage and complex moduli (up to 2X greater) than the young group throughout the length of the root. Dentin of the non-vital teeth exhibited significantly higher storage and complex moduli than that of the vital matched pairs. Both of the old groups of teeth exhibited significantly lower storage and complex modulus, as well as lower tan delta, than the root tissue of the young group. In summary, the radicular dentin loses its capacity for viscous deformation with aging and becomes embrittled as a consequence. This degradation is accentuated by treatments that involve removal of the pulp and appears to be most severe in the apical third of the root.

Acknowledgements

The authors gratefully acknowledge the comments and contributions of Dr. Ying Guo and Dr. Mark Mao of the Northwest Chinese Dental Association to this investigation. This work was partially supported by the Colgate-Palmolive Company, USA (PI D. Arola). The authors deny any conflicts of interest.

REFERENCES

- [1] Slade GD, Akinkugbe AA, Sanders AE. Projections of U.S. Edentulism prevalence following 5 decades of decline. *J Dent Res* 2014;93:959–65.
- [2] Cameron CE. The cracked tooth syndrome: additional findings. *J Am Dent Assoc* 1976;93:971–5.
- [3] Krusic JJ, Ritchie RO. Fatigue of mineralized tissues: cortical bone and dentin. *J Mech Behav Biomed Mater* 2008;1:3–17.
- [4] Seo D, Yi Y, Shin S, Park J. Analysis of factors associated with cracked teeth. *J Endod* 2012;38:288–92.
- [5] Cohen S, Berman LH, Blanco L, Bakland L, Kim JS. A demographic analysis of vertical root fractures. *J Endod* 2006;32:1160–3.
- [6] Lubisch EB, Hilton TJ, Ferracane J. Cracked teeth: a review of the literature. *J Esthet Restor Dent* 2010;22:158–67.
- [7] Nanci A. *Ten Cate's oral histology: development, structure, and function*. India: Elsevier; 2012.
- [8] Weber DF. Human dentine sclerosis: a microradiographic survey. *Arch Oral Biol* 1974;19:163–9.
- [9] Vasiliadis L, Darling AI, Levers BGH. The histology of sclerotic human root dentine. *Arch Oral Biol* 1983;28:693–700.
- [10] 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;26:7650–60.
- [11] Vasiliadis L, Stavrianos C, Dagkalis P, Parisi K, Stavriani I, Tassis D. Translucent root dentin in relationship to increasing age: review of the literature. *Res J Biol Sci* 2011;6:92–5.
- [12] Yahyazadehfar M, Ivancik J, Majd H, An B, Zhang D, Arola D. On the mechanics of fatigue and fracture in teeth. *Appl Mech Rev* 2014;66.
- [13] Arola D, Reprogl R. Effects of aging on the mechanical behavior of human dentin. *Biomaterials* 2005;26:4051–61.
- [14] Yan W, Montoya C, Øilo M, Ossa A, Paranjpe A, Zhang H, et al. Reduction in fracture resistance of the root with aging. *J Endod* 2017;43:1494–8.
- [15] Kinney JH, Nalla RK, Pople JA, Breunig TM, Ritchie RO. Age-related transparent root dentin: mineral concentration, crystallite size, and mechanical properties. *Biomaterials* 2005;26:3363–76.
- [16] Ivancik J, Neerchal NK, Romberg E, Arola D. The reduction in fatigue crack growth resistance of dentin with depth. *J Dent Res* 2011;90:1031–6.
- [17] Koester KJ, Ager III JW, Ritchie RO. The effect of aging on crack-growth resistance and toughening mechanisms in human dentin. *Biomaterials* 2008;29:1318–28.
- [18] Nazari A, Bajaj D, Zhang D, Romberg E, Arola D. Aging and the reduction in fracture toughness of human dentin. *J Mech Behav Biomed Mater* 2009;2:550–9.
- [19] Bajaj D, Sundaram N, Nazari A, Arola D. Age, dehydration and fatigue crack growth in dentin. *Biomaterials* 2006;27:2507–17.

- [20] Ivancik J, Majd H, Bajaj D, Romberg E, Arola D. Contributions of aging to the fatigue crack growth resistance of human dentin. *Acta Biomater* 2012;8:2737–46.
- [21] Tang W, Wu Y, Smales RJ. Identifying and reducing risks for potential fractures in endodontically treated teeth. *J Endod* 2010;36:609–17.
- [22] Adorno C, Yoshioka T, Jindan P, Kobayashi C, Suda H. The effect of endodontic procedures on apical crack initiation and propagation ex vivo. *Int Endod J* 2013;46:763–8.
- [23] Endodontists AA of. Endodontics: colleagues for excellence—Cracking the cracked tooth code 1997.
- [24] Ferracane JL, Funkhouser E, Hilton TJ, Gordan VV, Graves CL, Giese KA, et al. Observable characteristics coincident with internal cracks in teeth: findings from the National Dental Practice-based Research Network. *J Am Dent Assoc* 2018;149:885–92.e6.
- [25] Bürklein S, Tsotsis P, Schäfer E. Incidence of dentinal defects after root canal preparation: reciprocating versus rotary instrumentation. *J Endod* 2013;39:501–4.
- [26] Yoldas O, Yilmaz S, Atakan G, Kuden C, Kasan Z. Dentinal microcrack formation during root canal preparations by different NiTi rotary instruments and the self-adjusting file. *J Endod* 2012;38:232–5.
- [27] Shemesh H, Wesselink PR, Wu M-K. Incidence of dentinal defects after root canal filling procedures. *Int Endod J* 2010;43:995–1000.
- [28] Barreto MS, do Amaral Moraes R, da Rosa RA, Moreira CHC, Só MVR, Bier CAS. Vertical root fractures and dentin defects: effects of root canal preparation, filling, and mechanical cycling. *J Endod* 2012;38:1135–9.
- [29] De-Deus G, Silva EJNL, Marins J, Souza E, Neves A, de A, Gonçalves Belladonna F, et al. Lack of causal relationship between dentinal microcracks and root canal preparation with reciprocation systems. *J Endod* 2014;40:1447–50.
- [30] Haueisen H, Gärtner K, Kaiser L, Trohorsch D, Heidemann D. Vertical root fracture: prevalence, etiology, and diagnosis. *Quintessence Int (Berl)* 2013;44:467–74.
- [31] Thomas GJ, Whittaker DK, Embrey G. A comparative study of translucent apical dentine in vital and non-vital human teeth. *Arch Oral Biol* 1994;39:29–34.
- [32] Yan W, Montoya C, Øilo M, Ossa A, Paranjpe A, Zhang H, et al. Contribution of root canal treatment to the fracture resistance of dentin. *J Endod* 2019;45:189–93.
- [33] Ziskind D, Hasday M, Cohen SR, Wagner HD. Young's modulus of peritubular and intertubular human dentin by nano-indentation tests. *J Struct Biol* 2011;174:23–30.
- [34] Senawongse P, Otsuki M, Tagami J, Mjör I. Age-related changes in hardness and modulus of elasticity of dentine. *Arch Oral Biol* 2006;51:457–63.
- [35] Shepherd TN, Zhang J, Ovaert TC, Roeder RK, Niebur GL. Direct comparison of nanoindentation and macroscopic measurements of bone viscoelasticity. *J Mech Behav Biomed Mater* 2011;4:2055–62.
- [36] Balooch M, Demos SG, 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;12:507–14.
- [37] Ryou H, Romberg E, Pashley DH, Tay FR, Arola D. Nanoscopic dynamic mechanical properties of intertubular and peritubular dentin. *J Mech Behav Biomed Mater* 2012;7:3–16.
- [38] Ryou H, Romberg E, Pashley DH, Tay FR, Arola D. Importance of age on the dynamic mechanical behavior of intertubular and peritubular dentin. *J Mech Behav Biomed Mater* 2015;42:229–42.
- [39] Toledano M, Osorio E, Cabello I, Aguilera FS, López-López MT, Toledano-Osorio M, et al. Nanoscopic dynamic mechanical analysis of resin-infiltrated dentine, under in vitro chewing and bruxism events. *J Mech Behav Biomed Mater* 2016;54:33–47.
- [40] Toledano M, Osorio R, Osorio E, Cabello I, Toledano-Osorio M, Aguilera FS. In vitro mechanical stimulation facilitates stress dissipation and sealing ability at the conventional glass ionomer cement-dentin interface. *J Dent* 2018;73:61–9.
- [41] Toledano M, Osorio R, López-López MT, Aguilera FS, García-Godoy F, Toledano-Osorio M, et al. Mechanical loading influences the viscoelastic performance of the resin-carious dentin complex. *Biointerphases* 2017;12:021001.
- [42] Toledano M, Osorio E, Aguilera FS, Toledano-Osorio M, López-López MT, Osorio R. Stored potential energy increases and elastic properties alterations are produced after restoring dentin with Zn-containing amalgams. *J Mech Behav Biomed Mater* 2019;91:109–21. <http://dx.doi.org/10.1016/J.JMBBM.2018.12.002>.
- [43] Zhang Z, Mutluay M, Tezvergil-Mutluay A, Tay FR, Pashley DH, Arola D. Effects of EDC crosslinking on the stiffness of dentin hybrid layers evaluated by nanoDMA over time. *Dent Mater* 2017;33:904–14.
- [44] Ryou H, Pashley DH, Tay FR, Arola D. A characterization of the mechanical behavior of resin-infiltrated dentin using nanoscopic Dynamic Mechanical Analysis. *Dent Mater* 2013;29:719–28.
- [45] Bertassoni LE, Swain MV. Influence of hydration on nanoindentation induced energy expenditure of dentin. *J Biomech* 2012;45:1679–83.
- [46] Arola D. Fracture and aging of dentine. *J Biomater Dent* 2008;314–42.
- [47] 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;26:7650–60.
- [48] Burr DB. Changes in bone matrix properties with aging. *Bone* 2019;120:85–93.
- [49] 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.
- [50] Wang X, Shen X, Li X, Agrawal CM. Age-related changes in the collagen network and toughness of bone. *Bone* 2002;31:1–7.
- [51] Nyman JS, Roy A, Acuna RL, Gayle HJ, Reyes MJ, Tyler JH, et al. Age-related effect on the concentration of collagen crosslinks in human osteonal and interstitial bone tissue. *Bone* 2006;39:1210–7.
- [52] Walters C, Eyre DR. Collagen crosslinks in human dentin: increasing content of hydroxypyridinium residues with age. *Calcif Tissue Int* 1983;35:401–5.
- [53] Slutsky-Goldberg I, Maree M, Liberman R, Heling I. Effect of sodium hypochlorite on dentin microhardness. *J Endod* 2004;30:880–2.
- [54] Marending M, Luder HU, Brunner TJ, Knecht S, Stark WJ, Zehnder M. Effect of sodium hypochlorite on human root dentine – mechanical, chemical and structural evaluation. *Int Endod J* 2007;40:786–93.