Inelastic Deformation and Microcracking Process in Human Dentin

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ABSTRACT

Dentin is a mineralized collagen tissue with robust mechanical performance. Understanding the mechanical behaviour of dentin and its relations to the dentinal structure can provide insight into the design strategies to achieve tooth functions. This study focuses on the inelastic deformation of human dentin and its underlying mechanisms. By combining four-point bending tests with fluorescent staining and laser scanning confocal microscopy, it was found that human dentin, especially root dentin, exhibited significant inelastic deformation and developed extensive microdamage in the form of microcracks prior to fracture. Similar to bone, dense and wavy microcracks spread uniformly across the tensile surface of root dentin, while compressive microcracks formed cross-hatched patterns. The presence of peritubular dentin in coronal dentin dramatically decreased the extent of microcracking, reducing inelasticity. Dentinal tubules were found to be initiation sites of both tensile and compressive microcracks. A unique crack propagation process was observed in root dentin under tension: numerous ring-shaped cracks formed at each dentinal tubule ahead of a growing crack tip. The advance of the tensile microcrack occurred by the merging of those ring-shaped cracks. The current findings on the microcracking process associated with inelastic deformation helps to understand the nature of strength and toughness in dentin, as well as the mechanical significance for structural variations across the whole tooth.

Key words: Dentin, microcracking, inelastic deformation, toughness, laser scanning confocal microscopy.

INTRODUCTION

Dentin is a major structural component in human teeth. Biomechanically, it carries and transfers the masticatory loads from the enamel surface to the jaw bone (Nanci and Ten Cate 2008; Water 1980). To fulfill those functions over the lifetime of an individual, dentin and the whole tooth structure need to be mechanically robust. Understanding the mechanical behaviour of dentin and the detailed relations to the dentinal structure provide insight into the design strategies to achieve tooth functions and help to improve dental restoration techniques.

Structurally, dentin is a nanocomposite of collagen protein and carbonated apatite crystals (Currey 2002; Weiner and Wagner 1998). A distinctive feature in dentin is the dentinal tubules running from the pulp cavity to the periphery. The tubules are a few microns in diameter and occupy about 10% or less by volume of the bulk dentin (Marshall 1993; Nanci and Ten Cate 2008; Pashley 1996; Weiner and Wagner 1998). In human root dentin, dentinal tubules are surrounded by mineralized collagen fibrils organized layer by layer on planes (i.e., the incremental lines) transverse to the tubules (Jones 1984; Kramer 1951; Pashley 1996). In coronal dentin, the tubules are separated from the mineralized collagen fibrils (i.e., intertubular dentin) by a thin layer ($< 0.5 \mu\text{m}$) of highly mineralized peritubular dentin (Marshall et al. 1997; Weiner et al. 1999). There have been extensive mechanical studies in the literature on how such structural anisotropy and inhomogeneity in dentin affect the mechanical properties (Arola and Reprogl 2006; Arola et al. 2010; Inoue et al. 2012; Inoue et al. 2012; Ivancik et al. 2011; Ivancik and Arola 2013; Iwamoto and Ruse 2003; Kinney et al. 2003; Koester, et al. 2008; Nalla, et al. 2003; Wang 2005; Wang and Weiner 1998a; Wang and Weiner 1998b; Weiner and Wagner 1998; Yan et al. 2008). Briefly, the microhardness is isotropic (Wang and Weiner 1998a) while the elastic modulus, tensile strength, and fracture toughness depend on the orientation of structural elements such as the dentinal tubules (Inoue et al. 2012; Iwamoto and Ruse 2003; Kinney et al. 2003; Lertchirakarn et al. 2001; Nalla et al. 2003; Rasmussen et al. 1976; Soares et al. 2010). Coronal dentin is stiffer and harder than root dentin, but the latter is tougher and stronger in tension (Inoue et al. 2009; Plotino et al. 2007; Wang 2005; Yan et al. 2008). Various toughening mechanisms have been identified in dentin including crack deflection at incremental lines (Wang 2005), microcracking (Kahler et al. 2003; Koester et al. 2008;

Nalla et al. 2003), uncracked ligament bridging (Kahler et al. 2003; Koester et al. 2008; Nalla et al. 2003), as well as collagen fibril bridging (Nalla et al. 2003).

A mechanical phenomenon that has been far less addressed in the literature is the inelastic deformation in dentin. A good example is the typical tensile stress-strain curves reported by Sano et al. on both human and bovine dentin. The linear stress-strain stage is followed by a significant ($\sim 1-1.5\%$) non-linear, inelastic stage with minimum strain hardening (Sano et al. 1994). Other evidences of inelastic deformation in dentin can be found in reports by Jameson et al. (Jameson et al. 1993) and Arola and Reprogel (Arola and Reprogel 2006) on human dentin and by Brear et al. on narwhal tusk dentin (Brear et al. 1990). Such inelasticity is a strong indication of dentin's mechanical robustness. It is well-known in engineering that the capability of undergoing remarkable inelastic deformation increases the fracture resistance of a material through "process zone toughening" (Evans 1997). When sufficiently large, inelasticity enables a material to eliminate stress concentration at strain concentration sites (e.g., holes, corners, and flaws), rendering the material notch-insensitive (Evans and Zok 1994; Evans 1997). The possible involvement of inelasticity in dentin fracture was observed by Kahler et al. (Kahler et al. 2003). During crack opening, a fairly large zone ahead of the crack tip experienced structural dilation (as indicated by water ingress into dentin) while unloading happened at the crack wake (as indicated by water egress).

In order to better interpret dentin strength and toughness, there is a need to understand the nature of inelasticity in dentin. Unfortunately, little information is available in the literature on the deformation mechanisms in dentin. This topic has been well-studied in bone, another mineralized collagen tissue (Currey and Brear 1974; Ebacher et al. 2007; Ebacher et al. 2012; Mercer et al. 2006; Wang and Gupta 2011; Zioupos and Currey 1994; Zioupos et al. 1994; Zioupos et al. 2008). It was shown by Zioupos et al. (Zioupos et al. 1994) that deviation from linearity in the stress-strain curves was accompanied by the formation of fine microcracks in the bone matrix. The microcracking process also strongly interacts with bone's structural components such as the lamellae, the Haversian systems, and the osteocyte lacunae and canaliculi networks (Ebacher et al. 2007; Ebacher and Wang 2009; Ebacher et al. 2012; Mercer et al. 2006; Reilly and Currey 1999). It is therefore hypothesized that microcracking-associated inelastic deformation also happens in

dentin. However, the detailed process may be governed by dentin's unique structural features such as the dentinal tubules, the incremental lines, and the peritubular dentin. To verify these hypotheses, the present study compared the mechanical behaviours of human coronal and root dentin using bending tests followed by detailed microscopy analysis of the microcracking process.

MATERIAL AND METHODS

Four upper first premolars, extracted from two adolescents for orthodontics purposes were collected. The extracted teeth were free of carious lesions or fractures. After cleaning, they were stored at -20° until further process. The study was approved by the Clinical Research Ethics Review Board of the University of British Columbia.

Specimen preparation

All premolars were sectioned into three pieces (crown, buccal root, and lingual root) with a diamond saw (Isomet 1000, Buehler) under constant water irrigation. The sections were mechanically ground (Beta, Buehler) into twelve beam specimens (three per tooth; four per location) and polished with 6.0 µm and 1.0 µm diamond suspensions to final dimensions of 0.4 mm × 1.2 mm × 7 mm. Specimens' long axes had buccal-lingual orientation in the crown and apical-coronal orientation in the root. Specimens' thicknesses (0.4 mm sides) had pulp-enamel or pulp-cementum orientations, respectively (Fig 1). All specimens were stored in phosphate-buffered solution (0.1 mol; pH 7.4, 4°C) until testing.

Mechanical testing

The dentin specimens were loaded to fracture in four-point bending (1.5 mm loading span, 5.0 mm supporting span) under wet conditions using an electromechanic testing machine (Minimat Materials Tester 2000, 20 N load cell) at a crosshead speed of 0.1 mm min⁻¹. As dentinal tubules have radial orientation in root dentin, their distribution density is higher near the pulp cavity (Mjor and Nordahl 1996; Nanci and Ten Cate 2008). To account for this microstructural variation across the bending beams' thicknesses, buccal root specimens were loaded on the surface facing the cementum (root Group A), while lingual root specimens were loaded on the surface facing

the pulp cavity (root Group B). Crown specimens were loaded on the surface facing the enamel (Fig. 1). Two root specimens (one from each group) were unloaded prior to fracture in order to examine the early events of microcrack development during inelastic deformation. Time, load, and displacement were recorded. Bending strengths were calculated for the three groups (crown, root A, and root B) and statistically compared using ANOVA and Holm-Sidak t-test with a confidence level of 95% ($p < 0.05$).

Microcracking analysis

Following mechanical testing, all specimens were stained with fluorescein dye to examine the relations between microcracking and the dentinal structure. Fluorescein has been known for its capability of staining very fine microcracks in bone (Reilly and Currey 1999). Details of the staining protocol have been previously described (Ebacher et al. 2007). Briefly, the specimens were dehydrated in acetone and a graded series of ethanol solutions (80%, 90%, and 100%) for 24 h per step. They were finally immersed overnight in a filtered saturated solution of fluorescein (Fisher Scientific) and 70% ethanol.

The specimens were then observed under an epi-fluorescence microscope (Nikon Eclipse E600; excitation at ~490 nm and emission at ~525 nm). Selected sites were further examined using laser scanning confocal microscopy (LSCM; Zeiss LSM 780 or Olympus FluoView FV1000) to characterize the three-dimensional (3-D) morphologies of the microcracks and their interactions with the dentinal structure. Protocols for LSCM imaging were based on a previous study (Ebacher et al. 2012). An argon laser provided excitation at 488 nm and the emission occurred at 519 nm. The specimens were typically imaged from the surface down to depths of 10-12 μm with a step size of 100 to 500 nm depending on the magnification. Each series of 12-bits images thus obtained was reviewed, analyzed, and, when necessary, reconstructed in 3-D (Zen 2009 Light, Carl Zeiss MicroImaging GmbH; Imaris 7.6.0, Bitplane AG; ImageJ 1.43s, National Institutes of Health USA) to obtain microcrack dimensions and distribution.

Following microcracking characterization, two specimens (one crown, one root) were sputter-coated with a thin layer of gold and the fractured surfaces were imaged under a scanning electron microscope (SEM; Zeiss Sigma Schottky) at an accelerating voltage of 2 kV. Two other

specimens were ground and polished parallel to the tubules to 100 μm thin slices for polarized light observations of incremental lines in coronal and root dentin.

RESULTS

Load - deflection curves

The load - deflection curves from the four-point bending tests revealed very different mechanical responses for coronal and root dentin (Fig. 2). Coronal dentin showed a linear elastic stage followed by a short inelastic stage. Root dentin, however, had a much longer inelastic deformation stage. Overall, the flexural strength of root dentin was much higher than coronal dentin (326 MPa vs. 145 MPa). These results are slightly higher than that in the literature for root dentin but similar for coronal dentin (Plotino et al. 2007; Ryou et al. 2011). In root dentin, no statistically significant difference was observed between specimens bended towards the pulp cavity (group A) and those bended towards the cementum (group B) (Fig. 2b), even though the average strength in the latter group was slightly higher.

General fracture observations

Bending of coronal dentin resulted in a fracture plane transverse to the specimens' long axis and parallel to the tubules on the tensile side (Fig. 3a). This straight crack path continued to the compressive side where it changed to an oblique orientation (average angle of 42° to the long axis). SEM observations showed that the main fracture along the dentinal tubules often split the tubules into halves (Figs. 4a-b). The fracture also deflected between neighbouring tubules leaving fracture steps (Fig. 4a). In root dentin, the fracture on the tensile side was also roughly perpendicular to the specimens' long axis, but oblique to the tubules (average angle of 58° to the tubules). Under SEM, the fracture surface of root dentin was confirmed to be perpendicular to oblique to the dentinal tubules (Figs. 4c-d). The fracture on the compressive side was oblique to the specimen axis. Interestingly, this oblique path ran at a much smaller angle to the long axis than in coronal dentin, averaging 23° and reaching as low as 14° . Two of the six root specimens had a second oblique crack on the compressive side forming a "Y" shape with the main oblique

crack and the tensile crack (Fig. 3b). Such pattern corresponds to a “butterfly” bending fracture, typically observed in whole bone and cortical bone specimens (Ebacher et al. 2007). It suggests a difference in failure modes under tension and compression.

Microcracking observations

Root dentin under tension

Under the epi-fluorescence microscope, root dentin specimens showed bright green fluorescence on the tensile side indicating extensive microcracking of the dentinal tissue (Fig. 5). Microcracking was uniformly distributed across the inner loading span. The tensile damage zone extended beyond the central plane of the specimens’ thickness. Interestingly, the height of this tensile damage zone was greater in Group A (67.3% of the beam thickness when bended towards the pulp cavity) than in Group B (55.7% of the beam thickness when bended towards the cementum).

Under LSM, the damage on the tensile surface of root dentin appeared as dense wavy microcracks running transversally to the specimens’ long axis (and the tensile stress). They formed by the merging of clusters of dense microcrack arrays that initiated at the tubules (Fig. 6). The appearance of the microcrack arrays is very similar to those formed at blood vessels and osteocyte lacunae in bone (Reilly and Currey 1999). Although the tensile microcracks nucleated at the dentinal tubules on the tensile surface, side view scanning clearly showed that they did not propagate along the tubules (Fig 7). The densely packed microcracks initiated at the tensile surface and developed obliquely to the tubules through the thickness of the bending beams (Fig. 7a). Higher magnification observations revealed that the microcracks were homogeneously distributed within most of the intertubular dentin area, but often converged into discrete points along the tubules at proximity to the tubules (Fig 7b). Examination under polarized light found that tensile microcracks generally followed the direction of incremental lines in root dentin.

The propagation of tensile microcracks in root dentin involved an interesting process. Ahead of the crack growth front, ring-shaped cracks first formed around dentinal tubules (Fig. 8). Each of those rings was about 5-7 μm in diameter and oriented in a plane either perpendicular or slightly

oblique to the tubules. They were densely distributed along the tubules reaching an average inter-crack spacings of 1.4 μm in saturated regions (Figs. 8a-c). Most of the ring-shaped cracks around neighbouring tubules were initially separated from each other. However, some of them later extended and merged into longer cracks, which became part of the main tensile microcracks (Fig. 8). Obviously, the extension of tensile microcracks in root dentin specimens did not happen through the advance of an individual crack tip, but rather resulted from the coalescence of finer ring-shaped cracks ahead of the propagation front.

Root dentin under compression

The compressive surfaces of root dentin showed long and straight microcracks, oblique to the specimens' long axis (and the compressive stress) at about 40°. Two sets of such cracks intersected each other forming a "cross-hatched" cracking pattern, similar to the compressive microcracks found in bone (Fig. 9a) (Ebacher et al. 2007). LSCM examination provided details on the interaction between compressive microcracks and the dentin microstructure. Multiple cross-hatched microcracks formed at the tubules and developed into longer cracks through the tubules network in a shear process likely similar to that involving Haversian canals in bone (Ebacher et al. 2012).

Coronal dentin

Microcracking in coronal dentin was not as extensive as that in root dentin. Inspection with LSCM was unable to locate any microcracks on the compressive side. The tensile side, however, showed obvious damage in areas close to the main fracture. Microcracks nucleated at dentinal tubules on the tensile surface (Fig. 10). Different from the dense clusters of microcracks in root dentin, each tubule in coronal dentin would often initiate only two microcracks located on opposite sides of the tubule hole. The cracks grew perpendicular to the tensile stress (and the specimens' long axes) and merged with microcracks from neighbouring tubules. Closer examination revealed that most microcracks seemed to stop at the boundary between intertubular and peritubular dentin (arrowheads in Fig. 10). It is not clear whether the cracks initiated at this interface or the fluorescein dye failed to stain the cracks inside the highly mineralized peritubular dentin.

Side view LSCM of tensile microcracking in coronal dentin showed that microcracks initiated on the tensile surface and extended towards the compressive side for more than half the bending beams' thickness. These microcracks followed the general direction of the tubules, parallel to the main fracture (Fig. 11a). LSCM 3-D reconstruction revealed the detailed interactions of the microcracking front with the dentinal tubules (Fig. 11b). Tensile microcracks in coronal dentin did not always propagate along the tubules. They were rather frequently deflected to adjacent tubules, consistent with the deflection steps observed under SEM (Fig. 4a).

DISCUSSION

In this study, by combining bending tests with fluorescent staining and laser scanning confocal microscopy, it was demonstrated that human dentin, in particular root dentin, deformed through extensive microcracking prior to fracture. The observations on microcracking patterns clearly highlighted the different damage mechanisms under tensile and compressive stress states. The initiation of microcracks was often associated with dentinal tubules. Structural elements in dentin such as dentinal tubules, peritubular dentin, and the incremental lines directly contributed to the subsequent development of the microcracks. These findings help us to better understand the deformation and fracture process of dentin and human teeth.

Inelastic deformation and microcracking in dentin

Dentin belongs to the bone family of materials (Weiner and Wagner 1998). Both dentin and bone share the same building block, the mineralized collagen fibril, but differ in the way fibrils are organized. Such similarity is reflected in their mechanical behaviours. As for bone, the load-deflection curve of dentin shows a significant inelastic stage. This is especially true for root dentin (Fig. 2a). In the current study, LSCM observations further showed that dentin follows similar inelastic deformation mechanisms to those in bone. Under tension, root dentin developed extensive damage across the gauge section in the form of densely distributed wavy microcracks running transversally to the tensile stress (Figs. 6-7). Under compression, straight shear microcracks formed easily recognizable "cross-hatched" patterns (Fig. 9). These microcracking features are essentially the same as those found in bone under similar mechanical loading (Currey

et al. 1994; Ebacher and Wang 2009; Wang and Gupta 2011). It is well-accepted that bone yielding and post-yield deformation processes are accompanied by microcracks formation (Zioupos and Currey 1994; Ebacher and Wang 2009; Wang and Gupta 2011). Those microcracks may result from sliding, stretching or decohesion between mineralized collagen fibrils, or from sliding at the mineral/collagen interfaces (Wang and Gupta 2011). Therefore, microcracking and the associated irreversible sliding processes are also operating during dentin's inelastic deformation. Such inelastic deformation mechanisms would directly lead to an increase in fracture resistance through process zone toughening (Evans and Zok 1994; Evans 1997; Wang and Gupta 2011).

Although starting from the same building block of mineralized collagen fibrils, dentin and bone have totally different hierarchical structures. In bone, there are the lamellar structures, the cement lines, the osteocyte lacunae and canaliculi, as well as the Haversian canals and the blood vessels (Currey 2002; Currey 2012; Weiner and Wagner 1998). Dentin does not have those elements. Instead, it has the dentinal tubules, the peritubular dentin, and the incremental lines. These unique structures make the detailed microcracking process in dentin different from that in bone. From a mechanics point of view, both dentin and bone contain "defects". Those may act as crack initiation sites and lead to localized failure. The dentinal defects, i.e., the dentinal tubules, are small in size (1-3 μm) and homogeneously distributed (Marshall et al. 1997; Nanci and Ten Cate 2008). The defects in bone, i.e., the Haversian canals and the osteocytes lacunae, are much larger and heterogeneously distributed (Currey 2002). Such structural difference may account for the differences in microcracking at the macroscopic level. Microcracking was quite homogeneous across the tensile gauge section in root dentin (Fig. 5) while it is much more heterogeneous in bone (Ebacher et al. 2007).

Root dentin vs. coronal dentin

One of the key structural differences between coronal and root dentin is the presence of peritubular dentin in coronal dentin. This highly mineralized cylindrical layer lacks a collagen matrix, containing only a fine network of organic material (Bertassoni et al. 2012; Gotliv and Veis 2007; Weiner et al. 1999). Although peritubular dentin increases coronal dentin's elastic modulus as compared to root dentin, it decreases the strength and fracture toughness (Inoue et al. 2009;

Plotino et al. 2007; Wang 2005; Yan et al. 2008). In an earlier study, it was demonstrated that the higher toughness of root dentin is achieved by crack deflection at incremental lines (i.e., the interfaces between mineralized collagen fibril layers), while the presence of peritubular dentin creates additional weak orientations, making coronal dentin easier to fracture (Wang 2005). Those earlier observations were confirmed in the present study. Additionally, LSCM provided further details on crack nucleation in dentin. In root dentin, tubules acted as stress concentration sites and induced "cross-hatched" shear microcracks under compression (Fig. 9). Shear microcracking at most tubules could impart root dentin with inelasticity. However, the degree of inelastic deformation remains limited due to a low density of microcracks generated by such process. Under tension, dentinal tubules were also microcracks' nucleation sites. Densely packed wavy microcracks arrays initiated at each tubule (Figs. 6-7). Their number could not be assessed at the resolution of LSCM (in the order of 200 nm). Such response to external stresses in root dentin is very similar to the microcracking around osteocyte lacunae in bone (Reilly and Currey 1999; Mercer et al. 2006). Based on the observed microcracking processes and the mechanical properties, human root dentin behaves very similarly to bone. In coronal dentin where peritubular dentin is present around each tubule, the microcracking process changed dramatically. First, microcracking was absent on the compressive surface. Second, each tubule only initiated two cracks at opposite locations around the hole that experience the highest stress concentration under tension (Fig. 10). Therefore, the inelasticity imparted by microcracking was much lower than for root dentin. From a mechanical point of view, human coronal dentin differs from bone.

Crack propagation in root dentin

A very interesting process observed in this study was the development of tensile microcracks in root dentin. In the region ahead of a growing tensile crack tip, numerous ring-shaped cracks were found surrounding each tubule. The propagation of tensile microcracks occurred through merging of ring-shaped cracks from neighbouring tubules (Fig. 12). Such process is similar to the propagation of shear microcracks within a bone lamella, where multiple shear cracks initiate at osteocyte canaliculi ahead of the main crack tip and later merge with the main microcrack (Ebacher et al. 2012). The difference is that multiple ring-type cracks were formed along each

dentinal tubule presumably by tensile stress along the tubules. Since each dentinal tubule is surrounded by mineralized collagen fibrils laid down layer by layer along the tubule axes, local fluctuation may have led to high stresses and crack initiation. Multiple microcracks along the tubules suggest the presence of a bridging force along the tubular direction. This may arise from possible entangled collagen network within intertubular dentin. Preferred microcrack nucleation at dentinal tubules and its involvement in the advancement of a crack tip was reported earlier by Nalla et al. (Nalla et al. 2003). However, only a few microcracks were noted ahead of the crack front in that study, possibly due to the resolution limitation of SEM in detecting fine microcracks. The fluorescein staining and LSCM analyses in the current study suggest that microcracking at a crack tip could be very extensive.

The ring-shaped features around dentinal tubules have not been reported as cracks in the literature. However, they may have some clinical relevance. Their morphology looks similar to the alizarin red stained "bamboo-like" tubules observed in adult incisors extracted due to periodontal diseases or periapical infections (Kagayama et al. 1999). Another case with very similar ring-shaped feature around dentinal tubules was noted on the superficial coronal dentin layer of an artificial cavity coated with toluidine blue-loaded bonding agent during adhesive restoration simulations. In the histological observations, ring shaped structures were evident around dentinal tubules (Prof. Alejandro Oyarzún, personal communication). The possible link between these studies is a topic for future investigation.

CONCLUSIONS

Human root dentin has higher flexural strength and more significant inelastic deformation than coronal dentin. Inelastic deformation in dentin happens through microcracking. The morphology of microcracks in dentin strongly depends on the stress with tensile microcracks showing a dense wavy shape and compressive microcracks forming a typical cross-hatched pattern. Dentinal tubules directly facilitated both the initiation and propagation of microcracks. Other structural elements in dentin such as peritubular dentin and incremental lines also contributed to the extent and orientation of microcracking.

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REFERENCES

- Arola, D., Bajaj, D., Ivancik, J., Majd H., Zhang, D., 2010. Fatigue of biomaterials: hard tissues. *Int. J. Fatigue* 32 (9), 1400-1412.
- Arola,D., Reprogl,RK., 2006. Tubule orientation and the fatigue strength of human dentin. *biomaterials* 27 (9), 2131-2140.
- Bertassoni, L., Stankoska, K., Swain, M., 2012. Insights into the structure and composition of the peritubular dentin organic matrix and the lamina limitans. *Micron*. 43 (2-3), 229-236.
- Brear, K., Currey, JD., Pond, CM., Ramsay, MA., 1990. The mechanical-properties of the dentin and cement of the tusk of the narwhal monodon-monoceros compared with those of other mineralized tissues. *Arch. Oral Biol*. 35 (8), 615-621.
- Currey, JD., 2002. *Bones: structure and mechanics*. Princeton University Press, NJ.
- Currey, JD., Brear, K., 1974. Tensile yield in bone. *Calcif. Tissue Res*. 15, 173-179.
- Currey, JD., Brear, K., Zioupos, P., 1994. Dependence of mechanical-properties on fiber angle in narwhal tusk, a highly oriented biological composite. *J.Biomech*. 27 (7), 885-897.
- Currey, JD., 2012. The Structure and mechanics of bone. *J. Mater. Sci*. 47 (1), 41-54.
- Currey, JD., Zioupos, P., 2001. The effect of porous microstructure on the anisotropy of bone-like tissue: a counterexample. *J.Biomech*. 34 (5), 707-708.
- Ebacher, V., Guy, P., Oxland, TR., Wang, RZ., 2012. Sub-lamellar microcracking and roles of canaliculi in human cortical bone. *Acta Biomater*. 8 (3), 1093-1100.
- Ebacher, V., Tang, C., McKay, H., Oxland, TR., Guy, P., et al., 2007. Strain redistribution and cracking behavior of human bone during bending. *Bone* 40 (5), 1265-1275.

- Ebacher, V., Wang, RZ., 2009. A unique microcracking process associated with the inelastic deformation of haversian bone. *Adv. Funct. Mater.* 19 (1), 57-66.
- Evans, AG., 1997. Design and life prediction issues for high-temperature engineering ceramics and their composites. *Acta Mater.* 45 (1), 23-40.
- Evans, AG., Zok, FW., 1994. The physics and mechanics of brittle matrix composites. *solid state physics - advances in research and applications*, 47:177-286.
- Gotliv, BA., Veis, A., 2007. Peritubular dentin, a vertebrate apatitic mineralized tissue without collagen: role of a phospholipid-proteolipid complex. *Calcif. Tissue Int.* 81 (3), 191-205.
- Inoue, T., Saito, M., Yamamoto, M., Nishimura, F., Miyazaki, T., 2012. relation between incremental lines and tensile strength of coronal dentin. *Dent. Mater. J.* 31 (4), 541-548.
- Inoue, T., Saito, M., Yamamoto, M., Debari K., Kou K., et al., 2009. Comparison of nanohardness between coronal and radicular intertubular dentin. *Dent. Mater. J.* 28 (3), 295-300.
- Ivancik, J., Arola, D., 2013. The importance of microstructural variations on the fracture toughness of human dentin. *Biomaterials* 34 (4), 864-874.
- Ivancik, J., Neerchal, NK., Romberg, E., Arola, D., 2011. The reduction in fatigue crack growth resistance of dentin with depth. *J. Dent. Res.* 90 (8), 1031-1036.
- Iwamoto, N., Ruse, ND., 2003. Fracture toughness of human dentin. *J. Biomed. Mater. Res. Part A.* 66A (3), 507-512.
- Jameson, MW., Hood JAA., Tidmarsh, BG., 1993. The effects of dehydration and rehydration on some mechanical-properties of human dentin. *J. Biomech.* 26 (9), 1055-1065.
- Jones, SJ., 1984. Ultrastructure of dentin and dentinogenesis. Pp. 81-134 in *Dentin and dentinogenesis.*, edited by Linde, A. Boca Raton: CRC Press.
- Kagayama, M., Sasano, Y., Sato, H., Kamakura, S., Motegi, K., et al., 1999. Confocal microscopy of dentinal tubules in human tooth stained with alizarin red. *Anat. Embryol.* 199, 233-238.
- Kahler, B., Swain, MV., Moule, A., 2003. Fracture-toughening mechanisms responsible for differences in work to fracture of hydrated and dehydrated dentine. *J Biomech.* 36 (2), 229-237.
- Kinney, JH., Marshall, SJ., Marshall, GW., 2003. The mechanical properties of human dentin: a critical review and re-evaluation of the dental literature. *Crit. Rev. Oral Biol. Med.* 14 (1), 13-29.
- Koester, KJ., Ager, JW., Ritchie, RO., 2008. The effect of aging on crack-growth resistance and toughening mechanisms in human dentin. *Biomaterials* 29 (10), 1318-1328.

- Kramer, IRH., 1951. The distribution of collagen fibrils in the dentine matrix. *Br. Dent. J.* 91 (1), 1-7.
- Lertchirakarn, V., Palamara, JEA., Messer, HH., 2001. Anisotropy of tensile strength of root dentin. *J. Dent. Res.* 80 (2), 453-456.
- Marshall, GW., 1993. Dentin: microstructure and characterization. *Quintessence Int.* 24, 606-617.
- Marshall, GW., Marshall, SJ., Kinney, JH., Balooch, M., 1997. The dentin substrate: structure and properties related to bonding. *J. Dent.* 25(6):441-458.
- Mercer, C., He, MY., Wang, RZ., Evans, AG., 2006. Mechanisms governing the inelastic deformation of cortical bone and application to trabecular bone. *Acta Biomater.* 2 (1), 59-68.
- Mjor, IA., Nordahl, I., 1996. The Density and branching of dentinal tubules in human teeth. *Arch. Oral Biol.* 41 (5), 401-412.
- Nalla, RK., Kinney, JH., Ritchie, RO. 2003. Effect of orientation on the in vitro fracture toughness of dentin: the role of toughening mechanisms. *Biomaterials* 24 (22), 3955-3968.
- Nanci, A., Ten Cate, AR., 2008. *Ten Cate's oral histology, development, structure and function.* St. Louis, Mo.: Mosby Elsevier.
- Pashley, DH., 1996. Dynamics of the pulpo-dentin complex. *Crit. Rev. Oral Biol. Med.* 7 (2), 104-133.
- Plotino, G., Grande, NM., Bedinii, R., Pameijer, CH., Somma, F., 2007. flexural properties of endodontic posts and human root dentin. *Dent. Mater.* 23 (9), 1129-1135.
- Rasmussen, ST., Patchin, RE., Scott, DB., Heuer, AH., 1976. Fracture properties of human enamel and dentin. *J. Dent. Res.* 55, 154-164.
- Reilly, GC., Currey, JD., 1999. The development of microcracking and failure in bone depends on the loading mode to which it is adapted. *J. Exp. Biol.* 202 (5), 543-552.
- Ryou, H., Amin, N., Ross, A., Eidelman, N., Wang, DH., et al., 2011. Contributions of microstructure and chemical composition to the mechanical properties of dentin. *J. Mater. Sci.–Mater. Med.* 22 (5), 1127-1135.
- Sano, H., Ciucchi, B., Matthews, WG., Pashley, DH., 1994. Tensile properties of mineralized and demineralized human and bovine dentin. *J. Dent. Res.* 73 (6), 1205-1211.
- Soares, CJ., Castro, CG., Neiva, NA., Soares, PV., Santos, PCF., et al., 2010. Effect of gamma irradiation on ultimate tensile strength of enamel and dentin. *J. Dent. Res.* 89 (2), 159-164.

- Wang, RZ., 2005. Anisotropic fracture in bovine root and coronal dentin. *Dent. Mater.* 21 (5), 429-436.
- Wang, RZ., Gupta, HS., 2011. Deformation and Fracture Mechanisms of Bone and Nacre. *Ann. Rev. Mater. Res.* Vol 41 :41-73.
- Wang, RZ.; Weiner, S., 1998a. Human Root Dentin: Structural Anisotropy and Vickers Microhardness Isotropy. *Connect. Tissue Res.* 39(4):269-279.
- Wang, RZ.; Weiner, S., 1998b. Strain-structure relations in human teeth using moire fringes. *J. Biomech.* 31 (2), 135-141.
- Water, NE., 1980. Some mechanical and physical properties of teeth. Pp. 99-135. in *The mechanical properties of biological materials (Symposia of the society for experimental biology 34).*, edited by Vincent, CJ. London: Cambridge University Press.
- Weiner, S., Wagner, HD., 1998. The material bone: structure mechanical function relations. *Annu. Rev. Mat. Sci* 28:271-298.
- Weiner, S., Veis, A., Beniash, E., Arad, T., Dillon, JW., et al., 1999. Peritubular dentin formation: crystal organization and the macromolecular constituents in human teeth. *J. Struct. Biol.* 126 (1), 27-41.
- Yan, J., Taskonak, B., Platt, JA., Mecholsky, JJ. 2008. Evaluation of fracture toughness of human dentin using elastic-plastic fracture mechanics. *J. Biomech.* 41 (6), 1253-1259.
- Zioupou, P., Currey, JD., 1994. The extent of microcracking and the morphology of microcracks in damaged bone. *J. Mater. Sci.* 29 (4), 978-986.
- Zioupou, P.; Currey, JD.; Sedman, AJ., 1994. An examination of the micromechanics of failure of bone and antler by acoustic emission tests and laser scanning confocal microscopy. *Med. Eng. Phys.* 16:203-212.
- Zioupou, P.; Hansen, U.; Currey, JD., 2008. Microcracking damage and the fracture process in relation to strain rate in human cortical bone tensile failure. *J. Biomech.* 41, 2932-2939.

FIGURE LEGNEDS

Figure 1. Schematic of a human premolar and four-point bending specimens of coronal, root A (buccal or facial), and root B (lingual) dentin. The loading and supporting points are shown.

Figure 2. Typical load-deflection curves (a) and flexural strengths (b), for coronal, root A, and root B specimens.

Figure 3. Side views of typical fracture patterns in coronal dentin (a) and root dentin (b). The root specimen shows a “Y” pattern or “butterfly” fracture (arrowhead).

Figure 4. SEM images of fracture surface in coronal dentin (a, b) and root dentin (c, d).

Figure 5. Tensile microcracking in root dentin under epi-fluorescence microscopy: (a) Uniform microcracking across the tensile surface; (b) Side view of tensile microcracking extending past the central plane of the specimen thickness. The loading points of the four-point bending are shown.

Figure 6. LSCM imaging of tensile surface in root dentin showing wavy microcracking. Multiple microcracks nucleating at many dentinal tubules (arrowheads). A single tubule could initiate numerous microcracks, which connected to neighboring tubules. The orientation of the cracks is parallel to the fracture plane and therefore perpendicular to the beams' long axes and the tensile stress.

Figure 7. Side-view LCSM imaging showing tensile microcracking in root dentin. (a) Microcracks oriented oblique to dentinal tubules (running from upper right to the lower left) at the fracture site; (b) Microcracks occupy most of the intertubular dentin area, but merge into specific points (arrowheads) when approaching tubules (higher magnification of region in a). Tensile stress horizontal.

Figure 8. Side-view LSCM images of tensile microcracks in root dentin at depths of 0.96 μm (a), 2.40 μm (b), 4.80 μm (c), and corresponding 10 μm thick 3-D reconstruction (d). Ring-shaped cracks are evident around dentinal tubules (arrowheads in a and d mark the same cracks, note $\sim 180^\circ$ rotation in d). Note the interactions of the ring-shaped cracks (marked by the arrowheads in (a)) with the dentinal tubule at different depth (b, c). Some coalesced into longer tensile microcracks (arrows in a and d). Tensile stress horizontal.

Figure 9. Compressive cross-hatched microcracking in root dentin by epi-fluorescence microscopy (a) and LSCM (b). Multiple microcracks developed at the dentinal tubules, oblique to specimens' long axis and compressive stress (horizontal).

Figure 10. LSCM imaging of tensile surface showing microcrack initiating at tubules in coronal dentin. Each tubule could nucleate two microcracks in opposite directions, perpendicular to the specimens' long axes and tensile stress (horizontal). The microcracks eventually merged with those of neighboring tubules. Some seem to stop at the intertubular-peritubular dentin boundary (arrowheads).

Figure 11. Side view LCSM imaging of tensile microcracking in coronal dentin. (a) Tensile microcracks developed along the tubules at the fracture site; (b) Corresponding 3-D reconstruction showing microcrack deflected to adjacent tubules at the microcracking front. Tensile stress horizontal.

Figure 12. Schematic of tensile microcracking process in root dentin. Multiple ring-shaped cracks initiate around dentinal tubules (vertical white columns). These ring-shaped cracks later coalesce and merge with the advancing crack tip. The horizontally organized grey lines mark the incremental lines in dentin.

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