

Measurement of the Energy Absorbed during Nanoscale Deformation of Human Peritubular and Intertubular Dentin

Jiahau Yan^{1,2}, Burak Taskonak^{1,3}, John J. Mecholsky Jr.^{4*}

¹Department of Restorative Dentistry, Indiana University School of Dentistry, Indianapolis, IN, USA

²Corning Inc., Corning, NY, USA

³Private Practice, Fort Lauderdale, FL, USA

⁴Department of Materials Science and Engineering, College of Engineering, University of Florida, Gainesville, FL, USA Email: *jmech@mse.ufl.edu

How to cite this paper: Yan, J., Taskonak, B. and Mecholsky Jr., J.J. (2022) Measurement of the Energy Absorbed during Nanoscale Deformation of Human Peritubular and Intertubular Dentin. Materials Sciences and Applications, 13, 144-157. https://doi.org/10.4236/msa.2022.134010

Received: March 4, 2022 Accepted: April 22, 2022 Published: April 25, 2022

Copyright © 2022 by author(s) and Scientific Research Publishing Inc. This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).

http://creativecommons.org/licenses/by/4.0/ **Open Access**

٢

Abstract

Mineralized tissues are usually constructed of nanosized materials with ordered hierarchical structures. The main reason for their high load-bearing ability is the multi-scale hierarchy. It is important to have a method for measuring the energy absorbed during the nanoscale deformation of mineralized tissues. The objective of this study was to use a combination of nanoindentation and elastic-plastic mechanics techniques to measure the damage resistance of peritubular and intertubular dentin, based on the energy consumed in the plastic deformation regime and the volume created by the indents. The control materials were soda-lime glass, gold, and poly-methyl methacrylate (PMMA). Plastic deformation energy was calculated from the plastic part of load-displacement curves. The mean values of peritubular dentin and intertubular dentin were 3.8×10^9 , and 5.2×10^9 J/m³, respectively, compared to glass, PMMA, and gold which were 3.3×10^7 , 1.3×10^9 , and 3.1×10^9 J/m³, respectively. This method can be applied to study the resistance of mineralized tissues or organic/inorganic hybrid materials to deformation at the nanoscale.

Keywords

Dentin, Nano-Indentation, Plastic Deformation Energy, Elastic-Plastic Mechanics

1. Introduction

Biological hard tissues are composite materials incorporating both inorganic

components (e.g., phosphates and carbonates) and organic components (macromolecular structural units including proteins and polysaccharides). These materials have excellent mechanical properties mainly because of their hierarchically ordered structures from the nano-scale to the macro-scale [1] [2]. These composites are the source of inspiration for the design and processing of synthetic materials based on both their exquisite structure and biologically controlled processing [3]. Although there have been many studies investigating the mechanical properties of mineralized tissues on the macro scale, their mechanical behavior is still unknown on the nano scale. A method that measures the mineralized tissue's resistance to contact damage is presented in this paper to distinguish the difference in resistance to damage/deformation at peritubular and intertubular dentin.

The stiffness of an organic/inorganic biocomposite is similar to that of the mineral constituent. However, the deformation energy can be several orders of magnitude greater than that of the mineral [4]. Monolithic CaCO₃ shows a work of fracture that is ~3000 times less than that of the composite shell (nacre) [5]. We investigate in this study a mechanical property: the energy absorbed during the deformation of dentin. Dentin is a composite of carbonated hydroxylapatite crystals surrounded by an organic matrix at the nanoscale. Knowledge of the deformation mechanisms of dentin at many length scales is important to develop biomimetic restorative materials [6]. Previous researchers have tried to address the mechanism of the high toughness of biological ceramics from various points of view [7] [8] [9] [10] [11]. However, the question of why the structure of biological mineralized tissue starts at the nanoscale remains unclear.

Dentin as a Biological Structure

Dentin, the mineralized tissue forming the bulk of the tooth, lies between the enamel and the pulp chamber (Figure 1). It has a characteristic structure consisting of a hydrated matrix of type I collagen fibers (~30% by volume) that is reinforced with nanocrystalline hydroxylapatite (~45% by volume) and fluid $(\sim 25\%$ by volume) [12]. The mineral is distributed in the form of 40 to 50 nm diameter crystallites in a scaffold created by the collagen fibrils (50 - 100 nm in diameter). The inorganic mineral is thought to provide the strength and the collagen to provide the toughness [13]. The distinctive feature of the microstructure is the distribution of cylindrical dentin tubules [14]. These tubules are surrounded by a collar of highly mineralized peritubular dentin (~1 µm thick) and are embedded within a matrix of less mineralized collagen, called the intertubular dentin [15]. The tubules are the pathways of the formative odontoblast cells and, moving inward during the formation of the crown, the tubule numerical density increases 2 - 4 times with depth [12] [13]. The tubule lumens (0.5 - 1.0 µm in diameter) are surrounded or lined by a hypermineralized cylinder of apatite that is 0.1 to 0.5 µm in thickness (Figure 1). To our knowledge, it has not been established in previous studies if there is a difference in damage resistance between peritubular dentin and intertubular dentin.



Figure 1. Scanning electron micrograph of a cross-section of human dentin displaying peritubular (a) and intertubular (b) dentin [39].

Dentin can be analyzed at many length scales. At the nanostructural level, dentin can be studied as an aggregate of minerals and collagen [16]. At the microstructural level, dentin is composed of cylindrical tubules interpenetrating a partial matrix phase of type I collagen [17]. Finally, at the functional, or continuum level, dentin appears as a homogeneous substance that fills the space between enamel and pulp (**Figure 1**) [18].

Nature does not use strong polymeric fiber structure in an unoriented form, such as usually occurs in synthetic polymers in composites or plastics. Nature uses continuous polymer fiber reinforcements [19]. In the case of dentin, type I collagen fibers are used as continuous polymer fiber reinforcements [20]. Collagen is of particular interest because the chains are wound into triple helices of about 640 nm length which are hydrogen bonded into fibrils.

It is essential to further study biological organic/inorganic composites since the aforementioned research has not investigated the detailed role played by the controlled structural variations at the nanometer scale (e.g., bone, dentin, and nacre) in the physical and mechanical properties of these materials [21]-[34]. Biological systems are a rich source of inspiration for developing novel synthetic materials where structural control is established over a wide length scale range starting from the nanometer size [35] [36]. A more detailed appreciation of their structural design on their physical and mechanical properties can be deduced by studying them at all length scales [17]. The fundamental information about the purpose of their unique structural design can inspire materials scientists to design composites that have superior physical and mechanical properties relative to the synthetic materials available today [37].

Investigating this deformation mechanism in human dentin can lead to better knowledge for designing synthetic biomaterials in load-bearing applications. We present one method to quantify the resistance to deformation of materials within the peritubular and intertubular regions of dentin.

2. Materials and Methods

The peritubular dentin and intertubular dentin have different mineral contents and may exhibit different responses to local damage. To critically evaluate their respective behavior requires a technique that works at the nanoscale.

One method to measure resistance to damage is to calculate the energy absorbed during the plastic deformation process [38]. Figure 2 shows three commonly observed load-extension curves. For materials that show only elastic deformation, load-extension curves may be similar to the ones in Figure 2(a) and Figure 2(b). For materials having moderate yielding, their load-extension curves may be similar to the one in Figure 2(c). Dentin hydrated matrix of type I collagen fibers (~30% by volume) provides load-extension curves similar to the one in Figure 2(c) [39]. By this definition, macroscopically, damage resistance of materials having moderate yielding such as dentin can be determined by testing the specimens in tension or flexure and is estimated using the area under the load-displacement curve (energy input). However, this method cannot be applied to nanoscale measurements since a method for fabricating nanoscale tensile specimens is currently unavailable. To solve this problem, a proposed method based on nanoindentation is used in this study. Figure 3(a) depicts a schematic diagram of the nanoindentation process. The load (P) is applied through the cantilever and drives the indenter tip (e.g., diamond) to penetrate the sample. The indenter continues to go down until a preset maximum load (P_{max}) is reached, which creates the maximum penetration depth (h_{max}) in the sample. The indenter is then retracted and in the meantime, the elastic part of the deformation in the sample also recovers. In the end, only the plastic part of the original maximum deformation is observed. For materials with viscoelastic behavior, the recovery of deformation is time-dependent, *i.e.*, the sample continues to recover until all the viscoelasticity is released. Figure 3(b) shows the typical nanoindentation curves of two different materials under the same maximum load (P_{max}). Curve I is a typical curve for brittle materials (materials that fracture without significant plastic deformation), which has a much smaller



Figure 2. Three common load-extension curves of materials under tension or flexure. The ordinate, P, is the load usually reported in micronewtons. The abscissa, v, is displacement usually reported in nanometers. (a) Brittle fracture with no yielding. (b) Brittle fracture with small-scale yielding. (c) Moderate scale yielding before fracture [39].



Figure 3. Schematic diagram (a) of a typical nanoindenter and its components and typical load-displacement curves (b) of one sample with a brittle nature (curve I) and another one with much greater ductility (curve II).

enclosed area between the loading and unloading curves. On the other hand, curve II is a typical one for materials with moderate or high ductility, such as polymers, metals, and some mineralized tissues such as dentin. The curve has a much greater enclosed area between the loading and unloading curves. Figure 4 shows a more detailed process during the loading and unloading processes. The indenter penetrates the sample as the load increases. It reaches the maximum penetration (h_{max}) at P_{max} and then it retracts (unloading). During the unloading process, because of the elastic nature of materials, the compressed zone recovers, and the final depth of the indent (h_f) is often much less than h_{max} (Figure 4(b)). The enclosed areas between the loading and unloading curves directly correspond to the energy spent in the plastic deformation of the samples during the indentation process (Figure 4(a)). During the indentation process, for brittle materials, a majority of the energy is spent in elastic deformation; however, for ductile materials, a significant amount of energy is spent in plastic deformation. Therefore, as evidenced by the curves (Figure 3(b)), brittle materials have much less energy spent in the plastic deformation regime than the materials which are moderately or highly ductile. Another phenomenon observed during the indentation process is that soft materials have a greater volume of the indention impression, compared to hard materials that have a small volume of indention impression under the same load. We used the energy spent in the plastic deformation along with the volume of the indent to quantify how well a material resists plastic damage upon the formation of permanent imprints (the plastic deformation), which can be formulated using

$$T_{pl} = \frac{W_{pl}}{V_{indent}} \tag{1}$$

where T_{pl} describes how resistant the material is to permanent deformation, W_{pl} is the energy calculated using the enclosed area and V_{indent} the volume created by the indentation process. W_{pl} can be expressed by integrating the indentation load (*P*) from 0 to P_{max} along the final depth of the indent (Figure 4(a)) as follows:

$$W_{pl} = \int Pxdh - W_{el} \tag{2}$$



Figure 4. (a) The energy spent during the nanoindentation process can be categorized as plastic energy (W_{pl}) and elastic energy (W_{el}). (b) The indenter penetrates the sample and reaches the maximum penetration (h_{max}) at P_{max} . During the unloading process, the compressed zone recovers and the final depth of the indent (h_{l}) is often much less than h_{max} .

where the indentation load (*P*) is measured during the indentation. The energy required to create a unit volume of the indentation (T_{pl}) can be estimated using Equation (1). The volume created by the indentation process (V_{indent}), can be obtained from the cross-sectional area (A_s) of contact of the indentation and the height of the maximum penetration (h_t).

$$V_{indent} = Ah_f / 3 \tag{3}$$

2.1. Specimen Preparation and Testing

Nanoindentations were performed on human peritubular and intratubular dentin and soda-lime glass, gold, and poly-methyl methacrylate (PMMA). We made five indentations for each material. Soda-lime glass, gold, and poly-methyl methacrylate were chosen to serve as reference materials. Human mandibular third molar teeth are obtained from young adults (between 18 and 30 years old). Collection and use of human teeth were conducted under Indiana University Purdue University Indianapolis Internal Review Board (IRB) permit #0308-74. Cross-sectional slices of 2 mm thickness are dissected from the coronal section of the human dentin on each tooth between the dentinoenamel junction and pulp chamber and polished to a surface roughness between 50 and 80 nm using sequential grit sandpapers and abrasive particles. The polished specimens are then glued to a holder and mounted on a nanoindenter attached to an atomic force microscope (Nanoscope 3A, Veeco Corp. Santa Barbara, CA). Nano indentations were performed on peritubular dentin, intertubular dentin, glass, PMMA and gold using a 36-µN load by a nanoindenter (Nanoscope 3A, Veeco Corp. Santa Barbara, CA). A Berkovich diamond tip forming a triangular pyramid was used both as an imaging probe and an indenter. With the nano-indentation apparatus/AFM system, surface topography imaging and indentation were performed sequentially at the same position allowing the analysis of surfaces before and after the test. The volumes of the imprints were estimated using Section Analysis (Figure 5) to determine the depth and length of the side of each indent. Energy absorbed during deformation values were calculated



Figure 5. Illustration of the indentation geometry for an ideal conical indenter.

from load vs. displacement curves using the method described above. Indentations were performed in air. Indentations were carried out on the cross-sectioned dentin specimens on peritubular and intertubular dentin (Figure 1 and Figure 6).

2.2. Statistical Analysis

ANOVA analysis was used to assess any differences between the materials. Paired t-tests were used to compare the intertubular and peritubular dentin for differences in volume, energy, and toughness. All statistical analyses are based on a p value of 0.05.

3. Results

The energy spent in the plastic deformation of the samples during the indentation process (W_{pl}), the volume of indentation imprints (V_{indent}) and mean apparent toughness (T_{pl}) values are given in **Table 1** (Figure 7(a) & Figure 7(b)). Using a one-way ANOVA, the T_{pl} results of the three reference materials are significantly different (p < 0.0001). In addition, the T_{pl} of PMMA is significantly greater than that of glass (p < 0.0001). The T_{pl} of gold is statistically significantly greater than that of PMMA (p < 0.0001).

The load-displacement curves of glass, gold, and PMMA show different plasticity in the three materials. The mean energy spent on plastic deformation of glass, PMMA, and gold were 1.5×10^{-15} , 1.5×10^{-12} , and 8.1×10^{-13} J, respectively. Furthermore, the mean volume of indentation imprints on glass, gold, and PMMA were 4.6×10^{-23} , 1.1×10^{-21} , and 2.6×10^{-22} m³, respectively. The energy spent per volume (deformation resistance) values of glass, PMMA, and gold were 3.3×10^7 , 1.3×10^9 , and 3.1×10^9 J/m³, respectively. It was found that the deformation resistance of gold is 94 times that of glass and 2.4 times that of PMMA. The plastic regime and deformation resistance of gold and PMMA are much greater than that of glass (**Figure 7(b**)).

The mean energy spent in plastic deformation of peritubular dentin and intertubular dentin were 1.9×10^{-11} , and 3.0×10^{-11} J, respectively (**Table 2**) (**Figure 7(b**)). The mean volume of indentation imprints on peritubular dentin and intertubular dentin were 5.1×10^{-21} , and 5.9×10^{-21} m³, respectively. The



Figure 6. (a) Schematic of the intertubular dentin and peritubular dentin showing the approximate location of the nano indentations. (b) Image of the intertubular dentin and peritubular dentin showing a nano indentation at the intertubular dentin. The image width is approximately 10 microneters.

deformation resistance values of peritubular dentin and the intertubular dentin were 4.2×10^9 , and 5.6×10^9 J/m³, respectively (**Table 2**) (**Figure 7(c)**). Using a paired t test, the T_{pl} of the intertubular dentin is significantly greater than that of the peritubular dentin (p = 0.001).

Intertubular dentin had statistically greater energy spent in plastic deformation than peritubular dentin (p = 0.0017). In addition, the indentation imprint volume of intertubular dentin group had statistically greater values than peritubular dentin group (p = 0.0213). Furthermore, the deformation resistance values of intertubular dentin group were statistically greater than the peritubular group (p = 0.003) (**Table 2**).

4. Discussion

Macroscopically, mechanical tests are often made under tension or flexure. However, currently, the same measurements mostly, if not all, cannot be achieved at the nanoscale. Our goal was to develop an alternative method that



Figure 7. (a) Representative load-displacement curves of glass, gold and PMMA and energy consumed during elastic deformation (W_{el}) is shown in load-displacement curve of PMMA. (b) Energy consumed during plastic deformation (W_{pl}) of glass, gold and PMMA. (c) Representative load-displacement curves of peritubular dentin and intertubular dentin.

Table 1. Results of energy (W_{pl}), indent impression volume (V_{indent}) and toughness measurements (T_{pl}) for glass, PMMA and gold with standard deviations (S.D.). [n = 5 each material].

	Glass	PMMA	Gold
$W_{pl} \pm $ S.D. (J)	$1.5 \pm 0.2 \; (\times 10^{-15})$	$1.5 \pm 0.3 \; (\times 10^{-12})$	$8.1 \pm 1.5 (\times 10^{-13})$
$V_{indent} \pm S.D. (m^3)$	$4.6 \pm 0.6 \; (\times 10^{-23})$	$1.1 \pm 0.2 \; (\times 10^{-21})$	$2.6 \pm 0.4 \;(\times 10^{-22})$
$T_{pl} \pm$ S.D. (J/m ³)	$0.33 \pm 0.04 \; (\times 10^8)$	$13 \pm 3 \; (\times 10^8)$	31 ± 5 (×10 ⁸)

Table 2. Results of energy (W_{pl}), indent impression volume (V_{indent}) and permanent deformation resistance (T_{pl}) for intertubular and peritubular dentin with standard deviations (S.D.). [n = 5 for each material].

	Peritubular Dentin	Intertubular Dentin
$W_{pl} \pm$ S.D. (J)	$1.9 \pm 0.4 \; (\times 10^{-11})$	$3.0 \pm 0.5 \; (\times 10^{-11})$
$V_{indent} \pm S.D. (m^3)$	$5.1 \pm 1.3 (\times 10^{-21})$	$5.9 \pm 1.0 \; (\times 10^{-21})$
$T_{pl} \pm$ S.D. (J/m ³)	$3.8 \pm 0.3 \; (\times 10^9)$	$5.2 \pm 0.4 \; (\times 10^9)$

can be used to quantify the deformation resistance of materials and use it to compare the difference between intertubular and peritubular dentin. [9] used a similar method to study the effect of hydration on the energy dissipation as a function of the hardness of dentin.

In this study, the total energy spent during the indentation includes elastic (W_{el}) and plastic (W_{pl}) contributions. However, it is W_{pl} that leaves the permanent imprint after the indentation. Therefore, only W_{pl} was included in the toughness calculation. Our results show that W_{pl} of gold and PMMA are much greater than that of glass, which is in good agreement with general observation, *i.e.*, gold has the greatest ductility among the three materials and glass has the least [40]. However, we suggest that our current method should be regarded as a comparison tool rather than a predictive method since the property it measures should be explained as "energy consumed during the creation of permanent deformation under indentation".

A defining feature of dentin is that the size of the fillers (peritubular dentin) leads to a dramatic increase in interfacial area compared to traditional synthetic composites. In addition, the difference in deformation resistance between the nano-sized filler (peritubular dentin) and matrix (intertubular dentin) can cause the crack front to stop and deviate at the interface. However, if the content of the fillers is too high, the composites may become brittle. This has been observed in another similar biological composite, bone [41]. Currey showed that energy absorbed until fracture in bone increases as the mineral density increases up to 66.5% and then decreases as ash content keeps increasing. It is also shown in this study that the deformation resistance of peritubular dentin ($\sim 1 \mu m$ thick) is significantly smaller than that of intertubular dentin due to its high mineral content (**Table 2**) (**Figure 7(c)**). In a previous study, we observed that the fracture sur-

faces of peritubular dentin are much smoother than those of intertubular dentin (**Figure 1**) [39].

There are composition-related changes evident in the energy density for deforming a unit volume of dentin tissue between intertubular and peritubular dentin. This is because the amount of collagen and hydroxyapatite are different in both tissues. This compositional information can be implemented to design novel synthetic composite biomaterials. The impact of the nano-sized filler deformation resistance and the matrix deformation resistance on the composite's mechanical behavior can be quantitatively determined using the method described above.

Nano-structured composites such as dentin can exhibit strength and strain-to-failure that substantially exceeds that of micro- or macro-structured composites [37] [42]. Nanostructure in a composite will contain smaller defects compared to micro-structured composites. This can prevent early failure, lead-ing to nanocomposites with increased ductility. The absence of a valid method to measure deformation resistance at the nanoscale presents a challenge both for controlling and predicting the properties of nanocomposites.

The outcome of this project may serve as a foundation for future projects that will investigate the structure-mechanical behavior relationship of nanocomposites and bio-composites to serve as a model for synthetic biomaterials.

5. Conclusion

A method using nanoindentation to estimate the nanoscale plastic deformation resistance of materials was proposed based on the energy spent in creating permanent deformation. The difference in composition and structure between peritubular dentin and intratubular dentin is the probable cause for the difference in their plastic deformation resistance.

Conflicts of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Data will be made available upon reasonable request.

References

- Yan, J., Mecholsky, J.J. and Clifton, K.B. (2007) How Tough Is Bone? Application of Elastic-Plastic Fracture Mechanics to Bone. *Bone*, 40, 479-484. https://doi.org/10.1016/j.bone.2006.08.013
- [2] Willet, T., Josey, D., Lu, R.X., Minhas, G. and Montesano, J. (2017) The Micro-Damage Process Zone during Transverse Cortical Bone Fracture: No Ears at Crack Growth Initiation. *Journal of Mechanical Behavior of Biomedical Materials*, 74, 371-382. <u>https://doi.org/10.1016/j.jmbbm.2017.06.029</u>
- [3] Zhang, B., Han, Q., Zhang, J., Han, Z., Niu, S. and Ren, L. (2020) Advanced Bio-Inspired Structural Materials: Local Properties Determine Overall Performance. *Materials Today*, **41**, 177-199. <u>https://doi.org/10.1016/j.mattod.2020.04.009</u>

- [4] Calvert, P. (1992) Biomimetic Ceramics and Composites. *Mrs Bulletin*, 17, 37-40. <u>https://doi.org/10.1557/S0883769400046467</u>
- [5] Jackson, A.P., Vincent, J.F.V. and Turner, R.M. (1988) The Mechanical Design of Nacre. *Proceedings of the Royal Society of London Series B: Biological Sciences*, 234, 415-440. <u>https://doi.org/10.1098/rspb.1988.0056</u>
- [6] Sarikaya, M., Fong, H., Frech, D.W. and Humbert, R. (1999) Biomimetic Assembly of Nanostructured Materials. *Bioceramics*, 293, 83-97. <u>https://doi.org/10.4028/www.scientific.net/MSF.293.83</u>
- [7] Kamat, S., Su, X., Ballarini, R. and Heuer, A.H. (2000) Structural Basis for the Fracture Toughness of the Shell of the Conch *Strombus gigas*. *Nature*, **405**, 1036-1040. <u>https://doi.org/10.1038/35016535</u>
- [8] Menig, R., Meyers, M.H., Meyers, M.A. and Vecchio, K.S. (2001) Quasi-Static and Dynamic Mechanical Response of *Strombus gigas* (Conch) Shells. *Materials Science* and Engineering A: Structural Materials Properties Microstructure and Processing, 297, 203-211. <u>https://doi.org/10.1016/S0921-5093(00)01228-4</u>
- Bertassoni, L.E. and Swain, M.V. (2012) Influence of Hydration on Nanoindentation Induced Energy Expenditure of Dentin. *Journal of Biomechanics*, 45, 1679-1683. <u>https://doi.org/10.1016/j.jbiomech.2012.03.021</u>
- [10] Xie, Z. and Yao, H. (2014) Crack Deflection and Flaw Tolerance in "Brick-and-Mortar" Structured Composites. *International Journal of Applied Mechanics*, 6, Article ID: 1450017. <u>https://doi.org/10.1142/S1758825114500173</u>
- [11] Kataruka, A., Mendu, K., Okeoghene, O., Puthuvelil, J. and Akono, A.T. (2017) Microscopic Assessment of Bone Toughness Using Scratch Tests. *Bone Reports*, 6, 17-25. <u>https://doi.org/10.1016/j.bonr.2016.12.001</u>
- [12] Kinney, J.H., Balooch, M., Marshall, G.M. and Marshall, S.J. (1999) A Micromechanics Model of the Elastic Properties of Human Dentine. *Archives of Oral Biolo*gy, 44, 813-822. <u>https://doi.org/10.1016/S0003-9969(99)00080-1</u>
- [13] Marshall, G.W., Inai, N., Magidi, I.C.W., Ballouch, M., Kinney, J.H., Tagami, J. and Marshall, S.J. (1998) Dentin Demineralisation: Effects of Dentin Depth, pH and Different Acids. *Dental Materials*, 14, 383-383.
- [14] Kruzic, J., Nalla, R.K., Kinney, J.H. and Ritchie, R.O. (2003) Crack Blunting, Crack Bridging and Resistance-Curve Fracture Mechanics in Dentin: Effect of Hydration. *Biomaterials*, 24, 5209-5221. <u>https://doi.org/10.1016/S0142-9612(03)00458-7</u>
- [15] Kinney, J.H., Balooch, M., Marshall, G.W. and Marshall, S.J. (1993) Atomic-Force Microscopic Study of Dimensional Changes in Human Dentin during Drying. *Archives of Oral Biology*, **38**, 1003-1007. <u>https://doi.org/10.1016/0003-9969(93)90114-2</u>
- [16] Halgaš, R., Dusza, J., Kaiferová, J., Kovácsova, L. and Markovská, N. (2013) Nanoindentation Testing of Human Enamel and Dentin. *Journal Ceramics-Silikáty*, 57, 92-99.
- [17] Seyedkavoosi, S. and Sevostianov, I. (2019) Micromechanics of Dentin: Review. *Reviews on Advanced Materials and Technologies*, 1, 1-26. https://doi.org/10.17586/2687-0568-2019-1-1-1-26
- [18] Oyena, M.I. (2006) Nanoindentation Hardness of Mineralized Tissues. Journal of Biomechanics, 39, 2699-2702. <u>https://doi.org/10.1016/j.jbiomech.2005.09.011</u>
- [19] Calvert, P. (1994) Strategies for Biomimetic Mineralization. Scripta Metallurgica et Materialia, 31, 977-982. <u>https://doi.org/10.1016/0956-716X(94)90513-4</u>
- [20] White, S.N., Luo, W., Paine, M.L., Fong, H., Sarikaya, M. and Snead, M.L. (2001) Biological Organization of Hydroxyapatite Crystallites into a Fibrous Continuum

Toughens and Controls Anisotropy in Human Enamel. *Journal of Dental Research*, **80**, 321-326. <u>https://doi.org/10.1177/00220345010800010501</u>

- [21] Arola, D., Rouland, J.A. and Zhang, D. (2002) Fatigue and Fracture of Bovine Dentin. *Experimental Mechanics*, 42, 380-388. <u>https://doi.org/10.1007/BF02412142</u>
- [22] Arola, D. and Zheng, W. (2006) Hydration and Dynamic Fatigue of Dentin. *Journal of Biomedical Materials Research Part A*, 77, 148-159. https://doi.org/10.1002/jbm.a.30634
- [23] Arola, D.D. and Reprogel, R.K. (2006) Tubule Orientation and the Fatigue Strength of Human Dentin. *Biomaterials*, 27, 2131-2140. <u>https://doi.org/10.1016/j.biomaterials.2005.10.005</u>
- [24] El Mowafy, O.M. and Watts, D.C. (1986) Fracture Toughness of Human Dentin. Journal of Dental Research, 65, 677-681. https://doi.org/10.1177/00220345860650050901
- [25] Imbeni, V., Nalla, R.K., Bosi, C., Kinney, J.H. and Ritchie, R.O. (2003) In Vitro Fracture Toughness of Human Dentin. Journal of Biomedical Materials Research Part A, 66, 1-9. <u>https://doi.org/10.1002/jbm.a.10548</u>
- [26] Kahler, W., Swain, M.V. and Moule, A. (2003) Hydration Effects on Dentine Fracture Toughness and Crack Propagation. *Journal of Biomechanics*, 36, 229-237. <u>https://doi.org/10.1016/S0021-9290(02)00327-5</u>
- [27] Kruzic, J.J., Nalla, R.K., Kinney, J.H. and Ritchie, R.O. (2005) Mechanistic Aspects of *in Vitro* Fatigue-Crack Growth in Dentin. *Biomaterials*, 26, 1195-1204. <u>https://doi.org/10.1016/j.biomaterials.2004.04.051</u>
- [28] Nalla, R.K., Kinney, J.H. and Ritchie, R.O. (2003) On the Fracture of Human Dentin: Is It Stress- or Strain-Controlled? *Journal of Biomedical Materials Research Part A*, 67, 484-495. <u>https://doi.org/10.1002/jbm.a.10079</u>
- [29] Nalla, R.K., Kinney, J.H. and Ritchie, R.O. (2003) Effect of Orientation on the *in Vitro* Fracture Toughness of Dentin: The Role of Toughening Mechanisms. *Biomaterials*, 24, 3955-3968. <u>https://doi.org/10.1016/S0142-9612(03)00278-3</u>
- [30] Nalla, R.K., Kinney, J.H., Marshall, S.J. and Ritchie, R.O. (2004) On the *in Vitro* Fatigue Behavior of Human Dentin: Effect of Mean Stress. *Journal of Dental Research*, 83, 211-215. <u>https://doi.org/10.1177/154405910408300305</u>
- [31] Nalla, R.K., Kruzic, J.J. and Ritchie, R.O. (2004) On the Origin of the Toughness of Mineralized Tissue: Microcracking or Crack Bridging? *Bone*, 34, 790-798. <u>https://doi.org/10.1016/j.bone.2004.02.001</u>
- [32] Nalla, R.K., Balooch, M., Ager, J.W., Kruzic, J.J., Kinney, J.H. and Ritchie, R.O. (2005) Effects of Polar Solvents on the Fracture Resistance of Dentin: Role of Water Hydration. *Acta Biomaterialia*, 1, 31-43. <u>https://doi.org/10.1016/j.actbio.2004.08.002</u>
- [33] Yan, J. (2005) Elastic-Plastic Fracture Mechanics of Compact Bone. Ph.D. Dissertation, University of Florida, Gainesville.
- [34] Kahler, B., Kotousov, A. and Borkowski, K. (2005) On Fracture of Restored Teeth. Damage Assessment of Structures VI, 293-294, 245-251. https://doi.org/10.4028/0-87849-976-8.245
- [35] Baer, E., Hiltner, A. and Morgan, R.J. (1992) Biological and Synthetic Hierarchical Composites. *Physics Today*, 45, 60-67. <u>https://doi.org/10.1063/1.881344</u>
- [36] Kim, C.S., Randow, C. and Sano, T. (2015) Hybrid and Hierarchical Composite Materials. Springer, Berlin. <u>https://doi.org/10.1007/978-3-319-12868-9</u>
- [37] Zhang, X., Zhao, N. and He, C. (2020) The Superior Mechanical and Physical Properties of Nanocarbon Reinforced Bulk Composites Achieved by Architecture De-

sign: A Review. *Progress in Materials Science*, **113**, Article ID: 100672. <u>https://doi.org/10.1016/j.pmatsci.2020.100672</u>

- [38] Grellmann, W. (2001) New Developments in Toughness Evaluation of Polymers and Compounds by Fracture Mechanics. In: Grellmann, W. and Seidler, S., Eds., *Deformation and Fracture Behaviour of Polymers*, Springer, New York, 3-26. <u>https://doi.org/10.1007/978-3-662-04556-5_1</u>
- [39] Yan, J., Taskonak, B., Platt, J.A. and Mecholsky, J.J. (2008) Evaluation of Fracture Toughness of Human Dentin Using Elastic-Plastic Fracture Mechanics. *Journal of Biomechanics*, 41, 1253-1259. <u>https://doi.org/10.1016/j.jbiomech.2008.01.015</u>
- [40] Callister, W.D. (1999) Materials Science and Engineering: An Introduction. 5th Edition, John Wiley & Sons, New York, 792-801.
- [41] Currey, J.D. (1969) The Mechanical Consequences of Variation in the Mineral Content of Bone. *Journal of Biomechanics*, 2, 1-11. <u>https://doi.org/10.1016/0021-9290(69)90036-0</u>
- [42] Xia, Z., Riester, L., Curtin, W.A., Li, H., Sheldon, B.W., Liang, J., Chang, B. and Xu, J.M. (2004) Direct Observation of Toughening Mechanisms in Carbon Nanotube Ceramic Matrix Composites. *Acta Materialia*, **52**, 931-944. <u>https://doi.org/10.1016/j.actamat.2003.10.050</u>

Nomenclature

- P_{max} maximum applied indentation load
- *A* area of contact
- T_{pl} toughness of a material in resisting permanent deformation
- W_{pl} energy consumed to create a permanent deformation
- *V*_{indent} volume created by the indentation process
- $h_{\rm max}$ maximum penetration of indent
- h_f final depth of indent
- *P* indentation load