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Research Paper

The role of proteoglycans in the nanoindentation creep behavior of human dentin

Luiz E. Bertassoni[a,b,c,*], *Matheus Kury*[c], *Catherine Rathsam*[d], *Christopher B. Little*[e], *Michael V. Swain*[c,f]

[a]*Division of Biomaterials and Biomechanics, Department of Restorative Dentistry, School of Dentistry, Oregon Health and Science University, 2730 Moody Avenue, Collaborative Life Sciences Building, Level 6 N6005, Portland, 97201 OR, USA*

[b]*Center for Regenerative Medicine, Oregon Health and Science University, Portland, OR, USA*

[c]*Bioengineering Laboratory, Faculty of Dentistry, University of Sydney, Sydney, NSW, Australia*

[d]*Institute of Dental Research, Westmead Centre for Oral Health, NSW, Australia*

[e]*Raymond Purves Bone and Joint Research Labs, Kolling Institute of Medical Research, Royal North Shore Hospital, The University of Sydney, NSW, Australia*

[f]*Bioclinical Sciences Department, Faculty of Dentistry, University of Kuwait, Kuwait*

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ABSTRACT

Attempts to understand the mechanical behavior of dentin and other mineralized tissues have been primarily focused on the role of their more abundant matrix components, such as collagen and hydroxyapatite. The structural mechanisms endowing these biological materials with outstanding load bearing properties, however, remain elusive to date. Furthermore, while their response to deformation has been extensively studied, mechanisms contributing to their recovery from induced deformation remain poorly described in the literature. Here, we offer novel insights into the participation of proteoglycans (PG) and glycosaminoglycans (GAG) in regulating the nanoindentation creep deformation and recovery of mineralized and demineralized dentin. Accordingly, after the enzymatic digestion of either PGs and associated GAGs or only GAGs, the nanoindentation creep deformation of dentin increased significantly, while the relative recovery of both the mineralized and demineralized dentin dropped by 40–70%. In summary, our results suggest that PGs and GAGs may participate in a nanoscale mechanism that contributes significantly to the outstanding durability of dentin and possibly other mineralized tissues of similar composition.

*Corresponding author at: Division of Biomaterials and Biomechanics, Department of Restorative Dentistry, School of Dentistry, Oregon Health and Science University, 2730 Moody Avenue, Collaborative Life Sciences Building, Level 6 N6005, Portland, 97201 OR, USA.
E-mail address: bertasso@ohsu.edu (L.E. Bertassoni).

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1. Introduction

Studies concerning the regulation of deformation response in human mineralized tissues, including dentin and bone, have been primarily focused on the role of their more predominant constituents, namely collagen and hydroxyapatite. A number of nanoscale deformation mechanisms have been proposed to explain the effective load bearing ability of mineralized tissues, including void and crack formation (Gupta et al., 2005), mineralized collagen fibril-matrix shear (Burr et al., 1997), and nanogranular friction between mineral particles (Tai et al., 2006), among several other mechanisms (Fratzl, 2008a, 2008b; Sugiyama et al., 2007; Gupta et al., 2006; Jager and Fratzl, 2000; Koester et al., 2008). Despite these extensive efforts, the evidence of mineralized tissues' ability to *recover* from deformation, and thereby prevent successive accumulation of plastic or non-linear strain remains virtually non-existent in the literature.

Dentin is a load bearing mineralized composite that represents the largest component of tooth structure, sharing great compositional similarities to bone (Bertassoni et al., 2009, 2012). Its primary biomechanical role is to provide a tougher foundation for the brittle enamel (Imbeni et al., 2005), thus playing a major role in the durability of teeth. Similar to bone, dentin is primarily composed of type I collagen fibrils, water and nanocrystallites of carbonated hydroxyapatite (Bertassoni et al., 2012). Although these more abundant components have received considerable attention regarding their contributions to the mechanical properties of mineralized tissues, the specific role of the structures responsible for their mutual interaction in the matrix have been underestimated thus far, particularly the organic components interconnecting the collagen fibrils in the matrix.

Proteoglycans (PGs) and their glycosaminoglycan (GAGs) side chains represent a major group of noncollagenous structures with known structural and mechanical relevance (Goldberg and Takagi, 1993; Bertassoni and Swain, 2014; Bertassoni and Marshall, 2009) for vertebrates. Other matrix proteins, such as phosphoproteins and γ-carboxyglutamate-containing proteins are believed to be largely involved in mineral–matrix binding events (Linde, 1989), although they have also been associated with important mechanisms of dissipation of mechanical energy (Adams et al., 2008; Fantner et al., 2007). Decorin and biglycan are the two members of the small-leucine-rich-proteoglycan (SLRP) family predominantly expressed in dentin (Goldberg and Takagi, 1993). The GAGs most frequently found in dentin, in turn, are chondroitin 4-sulfate and a relatively lower content of chondroitin 6-sulfate (Goldberg and Takagi, 1993).

PGs have widely been shown to contribute extensively for nearly all connective tissues to resist against deformation under loading (Scott et al., 2004; Scott, 2003), particularly soft and non-minerlized tissues. Loss of specific PG family members has been linked to skin fragility, pulmonary emphysema, heart valve diseases, osteoarthritis (Melrose et al., 2008; Stanton et al., 2005; He and Swain, 2009) and several other conditions affecting the mechanical properties of multiple tissues (for a review refer to Ameye and Young (2002). Despite extensive evidence of the relevance of PGs to the extracellular matrix of nearly all vertebrates, knowledge of the mechanical role of these structures (Scott, 2003) in mineralized tissues, including dentin and bone, remains limited (Bertassoni et al., 2014; Xu et al., 1998). To address this knowledge gap we sought new insights into the roles of PGs and associated GAGs in the nanoindentation creep behavior of dentin. More specifically, we tested the hypothesis that, since PGs and GAGs interconnect collagen fibrils in dentin, they would function as key regulators of the viscoelastic response of healthy and demineralized dentin by hindering nanoindentation creep deformation and regulating deformation recovery.

2. Materials and methods

2.1. Specimen preparation and demineralization

Permanent and healthy human third molars ($N=8$) were obtained according to protocols approved by the Sydney South West Area Health Service and the Royal Prince Alfred hospital's bioethics committee on human research. Extracted teeth were thoroughly cleaned, rinsed in saline with 0.1% thymol and stored at room temperature. Roots and the top of the enamel crowns were cut using a low speed saw (Isomet; Buehler, Lake Bluff, IL, USA) under water irrigation in the mid-coronal region perpendicular to the tubule direction, thus exposing the remaining dentin on both sides. Dentin blocks measuring 3.5 mm in length and width and 2 mm in thickness were cut and subsequently ground and polished on silicon carbide papers of 600 and 1200 grits, followed by 9 μm and 1 μm polishing diamond paste on a rotary polishing machine (Buehler, Lake Bluff, IL, USA). Each specimen surface was partially covered with a masking tape to provide a reference area of mineralized dentin and the remaining surfaces were exposed to a demineralizing solution of 10% (v/v) citric acid for 2 min following demineralization protocols described elsewhere (Balooch et al., 2008).

2.2. Enzymatic digestions with chondroitinase-ABC and trypsin

Following demineralization, specimens were rinsed thoroughly with deionized water and the masking tape was removed. One set of specimens was immersed in a chondroitinase-ABC (C-ABC) solution ($N=4$) and another set was immersed in a trypsin solution ($N=4$). C-ABC was used to digest the chondroitin/dermatan sulfate GAG side-chains of PGs (Ho et al., 2005). Trypsin, on the other hand, was used primarily to cleave the protein core of proteoglycans following previously established protocols (Rapraeger and Bernfield, 1985). C-ABC solution contained 0.1 U/mL C-ABC from *Proteus vulgaris* (Sigma-Aldrich, St. Louis, MO), 50 mM Tris, 60 mM sodium acetate and 0.02% (w/v) bovine serum albumin at pH 8.0. Specimens were stored for 48 h in 37 °C under constant stirring and solutions were exchanged after 24 h. Trypsin solution contained 1 mg/ml TPCK-treated trypsin (Sigma–Aldrich, St. Louis, MO) with 0.2 M ammonium bicarbonate at pH 7.9. Specimens were stored for 48 h at 37 °C under constant stirring and solutions were also exchanged after 24 h. As our trypsin and C-ABC treatments aimed primarily at