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Effect of proteoglycans at interfaces as related to location, architecture, and mechanical cues

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ABSTRACT

Introduction: Covalently bound functional GAGs orchestrate tissue mechanics through time-dependent characteristics.
Objective: The role of specific glycosaminoglycans (GAGs) at the ligament–cementum and cementum–dentin interfaces within a human periodontal complex were examined. Matrix swelling and resistance to compression under health and modeled diseased states was investigated.
Materials and methods: The presence of keratin sulfate (KS) and chondroitin sulfate (CS) GAGs at the ligament–cementum and cementum–dentin interfaces in human molars ($N = 5$) was illustrated by using enzymes, atomic force microscopy (AFM), and AFM-based nanoindentation. The change in physical characteristics of modeled diseased states through sequential digestion of keratin sulfate (KS) and chondroitin sulfate (CS) GAGs was investigated. One-way ANOVA tests with $P < 0.05$ were performed to determine significant differences between groups. Additionally, the presence of mineral within the seemingly hygroscopic interfaces was investigated using transmission electron microscopy.
Results: Immunohistochemistry ($N = 3$) indicated presence of biglycan and fibromodulin small leucine rich proteoglycans at the interfaces. Digestion of matrices with enzymes confirmed the presence of KS and CS GAGs at the interfaces by illustrating a change in tissue architecture and mechanics. A significant increase in height (nm), decrease in elastic modulus (GPa), and tissue deformation rate (nm/s) of the PDL-C attachment site (215 ± 63–424 ± 94 nm; 1.5 ± 0.7–0.4 ± 0.2 GPa; 21 ± 7–48 ± 22 nm/s), and cementum–dentin interface (122 ± 69–360 ± 159 nm; 2.9 ± 1.3–0.7 ± 0.3 GPa; 18 ± 4–30 ± 6 nm/s) was observed.
Conclusions: The sequential removal of GAGs indicated loss in intricate structural hierarchy of hygroscopic interfaces. From a mechanics perspective, GAGs provide tissue recovery/resilience. The results of this study provide insights into the role of GAGs toward conserved tooth movement in the socket in response to mechanical loads, and modulation of potentially deleterious strain at tissue interfaces.

1. Introduction

In load bearing organs, several structural elements interact at various hierarchical length-scales. Interactions over length scales include microscale structural elements within cells that interface with matrix proteins through connective networks, which in turn maintain macroscale organ function. However, within this broad classification, there exists the need for a finer discretization to investigate the interplay of two dominant and distinctly shaped structural elements; fibrillar collagen and nonfibrillar proteoglycans (PGs). PGs interact with collagen and maintain mechanical and structural integrity of tissues (Ho, Sulyanto, Marshall, & Marshall, 2005), specifically at the attachment site of soft and hard tissues of a load bearing organ such as the dentoalveolar complex.

PGs act as macro-molecular struts between collagen fibrils and provide time-dependent characteristics identified as a shape

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memory property of tissues and their interfaces. Load-dampening characteristics of softer tissues are also maintained by the molecular integrity within collagen fibrils and covalently bonded PGs. When chemically bound, the two structurally and chemically different macromolecules provide load-dampening and tissue resilience characteristics. Resilience is often indicated by the ability of tissues to recover in size and shape over time upon load removal, and provides the tissue with shape memory. The time-dependent response to load is the viscous behavior of tissues. In the bone–ligament–tooth fibrous joint, the ligament is biphasic as it is a combination of fluid and solid phases. The fluid-like properties aid in lubrication, nutrient delivery, and hydrostatic pressure, all of which maintain organ vitality, and the ability of PDL to dampen dynamic loads. At healthy attachment sites, cohesive and adhesive bonds between structurally dissimilar macromolecules remain intact despite concentration differences, and natural turnover of different molecules. However, intact bonds between structural elements do not prevail under diseased or chronic inflammatory conditions (Embery, Waddington, Hall, & Last, 2000), and this in turn could change the time related response of tissues to mechanical loads. Thus, there lies the need to understand the effect of deteriorated PGs on organ function, specifically at the earlier stages of deterioration.

Higher and lower molecular weight PGs also known as non-aggregating and aggregating types form covalent bonds with collagen (Seog et al., 2002). These macromolecules are thought to be key players in absorbing dynamic mechanical loads, specifically within the collagen-rich soft tissue and at attachment sites that are an integral part of an organ. Within these tissues that predominantly contain type I collagen, the higher molecular weight PGs retain hydrostatic pressure and gross shape characteristics (Seog et al., 2002 Scott, 1988; Scott & Haigh, 1988), while lower molecular weight PGs within the same tissues and their adjacent interfaces are thought to maintain site-specific biophysical and biochemical environment for cells. Site-specific activities for cells promote and maintain local levels of organic and inorganic ratios at the ligament–bone and ligament–cementum attachment sites. In the human periodontium, the local levels of organic to inorganic components are predominantly reflected in precementum and osteoid layers relative to the respective bulk tissues that form the bone–PDL–tooth complex.

In the human periodontal complex, the prevalent PG families are of a lower molecular weight and belong to a family known as the small leucine rich proteoglycans (SLRPs). The protein cores of SLRPs have a mass between 45 and 55 kDa, leucine-rich repeats (LRRs) and typically contain covalently bound functional glycosaminoglycans (GAGs). Although the protein core of SLRPs binds directly to collagen, it is the associated GAGs that orchestrate several tissue characteristics including fibrillogenesis, spacing of the fibrils, and subsequent mineralization, thus modulating overall tissue mechanics (Scott, 1988). PGs have been identified within mineralized tissues as well as the mineral-free periodontal ligament (PDL) (Ababneh, Hall, & Embery, 1998; Ababneh, Hall, & Embery, 1999; Ho, Marshall, Ryder, & Marshall, 2007; Matheson, Larjava, & Hakkinen, 2005). PGs were also identified at the PDL–bone, PDL–cementum interfaces (Ho et al., 2010; Hurng et al., 2011; Lukinmaa, Mackie, & Thesleff, 1991). Previous studies from our laboratory have identified hygroscopic regions of the PDL–bone, PDL–cementum interfaces alluding to the presence of SLRPs (Ho et al., 2010; Hurng et al., 2011) and subsequently have identified SLRPs at the attachments sites (Chiu et al., 2012). Additionally, the presence of chondroitin sulfated GAGs was confirmed at a similar region, but at an interface between mineralized tissues, cementum and dentin within the complex of human molars (Ho, Balooch et al., 2004) (Yamamoto et al., 1999). Chondroitin-sulfated GAGs were also thought to be responsible for retaining radial fibrillar structure and mechanical properties of interfaces (Ho et al., 2005; Scott, 1988; Scott and Haigh, 1988; Scott and Stockwell, 2006; Yamamoto et al., 1999; Yamamoto, Domon, Takahashi, Islam, & Suzuki, 2000). In this study, we present two specific anatomical locations within a tooth that continue to be hypothesized as strain amplification sites (Qian, Todo, Morita, Matsushita, & Koyano, 2009; Ho et al., 2013). These include, (1) attachment sites (entheses) and interfacial zones where the pure organic PDL changes to mineralized cementum through PDL-inserts (also known as Sharpey's fibers) held together by cementum layer; (2) the cementum–dentin interface which was described as PDL-inserts attaching with root dentin forming a hygroscopic region commonly known as the CDJ (Ho, Balooch et al., 2004). A conceivable challenge to address the objective is the accessibility of attachment sites and the relevance of site-specific physical properties within the context of organ mechanics. In this study, the aforementioned challenge will be addressed through a commonly sought reductionist approach. The approach will include isolation of specific regions of interest in order to investigate the loss in tissue recovery characteristic due to breakdown of macromolecular tags, the PGs, and results will be discussed within the context of organ function. Thus, the objectives of this study are to investigate the crucial influence of GAGs, especially keratan sulfate and chondroitin sulfate at the PDL–cementum and cementum–dentin interfaces including cementum and dentin of the bone–ligament–tooth fibrous joint. Although a few studies have proposed CS containing SLRPs and high molecular weight PGs (such as lumican, versican, aggrecan, etc.) resist compressive and shear forces (Ho et al., 2007; Scott and Stockwell, 2006), little to no information about the overall mechanical influence of these biochemical moieties that aid in tissue recovery nature exists.

2. Materials and methods

2.1. Specimen preparation for immunohistochemistry

Extracted molars ($N = 3$) were coarsely sectioned and fixed in sodium phosphate-buffered (pH 7.0) 4% formaldehyde for 3 days. Specimens were demineralized using immunocal (Decal Chemical Corporation, Tallman, NY) formic acid solution for eight weeks, and were considered demineralized when addition of saturated ammonium oxalate to the solution failed to produce a precipitate.

2.1.1. Paraffin sections on microscope slides

Following dehydration with 80%, 95%, and 100% Flex alcohol (Richard-Allan Scientific, Kalamazoo, MI), the specimens were embedded in paraffin (Tissue Prep-II, Fisher Scientific, Fair Lawn, NJ). 5–6 μm thickness for histology sections was achieved with a rotary microtome (Reichert-Jung Biocut, Vienna, Austria) using disposable steel blades (TBF™ Inc., Shur/Sharp™, Fisher Scientific, Fair Lawn, NJ). The paraffin serial sections were mounted on Superfrost Plus microscope slides (Fisher Scientific, Fair Lawn, NJ).

2.1.2. Localizing KS- and CS-GAGs within the roots of human molars

Previously detailed (Ho et al., 2010) immunohistochemistry procedure was used to locate biglycan (BGN) and fibromodulin (FMOD) within cementum, the cementum dentin interface, and dentin. The respective antibodies for BGN and FMOD staining were obtained from Dr. Larry Fisher (NIDCR/NIH, Bethesda, MD), and are all polyclonal rabbit sera. Finally, 3,30-diaminobenzidine (DAB) enhanced liquid substrate system (Sigma, St. Louis, MO) was used per manufacturer's instructions with an incubation of 1 h to provide a brown coloration for epitope identification. An Olympus BX51 light microscope was used to characterize the slides using Image Pro software (Media Cybernetics Inc., Bethesda, MD).