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The plastic nature of the human bone–periodontal ligament–tooth fibrous joint

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ABSTRACT

This study investigates bony protrusions within a narrowed periodontal ligament space (PDL-space) of a human bone–PDL–tooth fibrous joint by mapping structural, biochemical, and mechanical heterogeneity. Higher resolution structural characterization was achieved via complementary atomic force microscopy (AFM), nano-transmission X-ray microscopy (nano-TXM), and microtomography (MicroXCT™). Structural heterogeneity was correlated to biochemical and elemental composition, illustrated via histochemistry and microprobe X-ray fluorescence analysis (μ-XRF), and mechanical heterogeneity evaluated by AFM-based nanoindentation. Results demonstrated that the narrowed PDL-space was due to invasion of bundle bone (BB) into PDL-space. Protruded BB had a wider range with higher elastic modulus values (2–8 GPa) compared to lamellar bone (0.8–6 GPa), and increased quantities of Ca, P and Zn as revealed by μ-XRF. Interestingly, the hygroscopic 10–30 μm interface between protruded BB and lamellar bone exhibited higher X-ray attenuation similar to cement lines and lamellae within bone. Localization of the small leucine rich proteoglycan biglycan (BGN) responsible for mineralization was observed at the PDL–bone interface and around the osteocyte lacunae. Based on these results, it can be argued that the LB–BB interface was the original site of PDL attachment, and that the genesis of protruded BB identified as protrusions occurred as a result of shift in strain. We emphasize the importance of bony protrusions within the context of organ function and that additional study is warranted.

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Introduction

Operating under Wolff's principles of organ use and disuse, the bone–periodontal ligament (PDL)–tooth fibrous joint and its respective tissue components react to occlusal inputs, that is, physiological and/or non-physiological loads. As such, the tissues adapt to functional demands, a cycle which may continue in perpetuity under prolonged loading. It is proposed that optimal function of the system is preserved by a characteristic functional PDL-space of 150–380 μm [1], maintained via balance of anabolic and catabolic activity of bone, and equivalent turnover of the PDL and lamellar cementum deposition [2] in response to the stress and strain fields generated during function.

Ideally, the occlusal loads placed on the tooth should result in the external architecture of the tooth to form a geometric conformity with bone and vice versa, under loaded conditions. The tooth is subjected to a variety of loads and when the majority of the compressive loads align with the anatomical axis of the tooth, the PDL could undergo shear, a mixed mode of tension and compression strain fields, supplemented by flexural moments at the tethered ends of the ligament, i.e. the radial PDL-inserts within bone and cementum [3]. As described by D'Arcy Thompson, force fields within organs and tissues promote the internal architecture [4]. In addition, Wolff's fundamental law of bone remodeling states that changes in the internal architecture of bones, when pathophysiologically altered (e.g. via extraneous loading or increased frequency of loading), can change the overall form of bone [5]. Bone cells are capable of adapting to local mechanical stresses via feedback loops, remodeling, and modeling micro and macro-structural changes

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to remain "functional", despite existing in a pathological state [5]. As a result, it seems inevitable that tissues and their interfaces adapt towards a pathological state to accommodate the significant shifts in loads and frequencies.

Previous studies have demonstrated, at a macroscale, heterogeneous composition in the form of physicochemical gradients within normal bone–PDL–tooth specimens [6]. It has been proposed that the graded interfaces within a joint consisting of uniform PDL-space can optimize distribution of functional loads, thus promoting functional life of the organ. Interestingly, in our recent study we demonstrated the presence of mechanical, structural, and chemical discontinuities (abrupt transitions between two dissimilar materials), localized at the alveolar PDL-bone and PDL-cementum interfaces within the complex [6]. Sharp gradients in the form of elastic discontinuities were observed within bone at the lamellar and protruded bone interface. These protruded sites manifesting as elastic discontinuities can be regions of high stress localization in a load-bearing complex. As such, the complex is conducive to amplified strains and stresses leading to perpetuation of the discontinuous interfaces while still maintaining function. Results in this study also raise the question that if organs are predisposed with discontinuities, should therapeutic loads be imposed? While functional load adaptation has been described in skeletal orthopedics [7–12], to our knowledge there exist no studies on bone–PDL–tooth fibrous joint adaptations within oral and craniofacial orthopedics.

The goal of this study is to illustrate characteristics of the narrowed PDL space within the bone–PDL–tooth complex. In addition, the elastic graded properties of the inter-lamellae, and lamellae of lamellar bone, bundle-lamellar bone interface, and bundle bone of a fibrous joint will be reported within the context of organ function by using erupted and undiseased specimens extracted from humans. This is because; a combination of matrix structure, biochemical and elemental composition, and elastic modulus of various tissues could affect biomechanical function. Hence, detailed PDL–bone, PDL–cementum, and protruded bone-lamellar bone integration at the 5–50 μm wide reduced complex will be discussed based on evaluations using various complementary higher resolution characterization techniques, such as atomic force microscopy (AFM), nano-X-ray computed tomography (nano-TXM), microprobe X-ray fluorescence, and an AFM-based nanoindentation technique.

Materials and methods

Specimens for MicroXCT™ imaging and physicochemical characterization

Specimens (N = 10) were acquired from patients undergoing orthodontic treatment where teeth along with proximal bone were removed. Teeth were removed due to crowding of dentition. Inclusion criterion for a specimen was proximal bone in the coronal half of the extracted tooth. Exclusion criteria were specimens with caries, periodontal disease, and/or root resorption. The protocol was approved by the UCSF Committee on Human Research. Specimens were sterilized using 0.31 Mrad of γ-radiation [13] and each specimen was scanned using a micro-X-ray computed tomography unit (μ-XCT, MicroXCT-200, Xradia, Pleasanton, CA) [3]. The mandibular–molar complex was then imaged using a μ-XCT [3] to identify tooth-alveolar bone association when compressed with a finite load. Five specimens were used for immunohistochemistry and histology. Another five specimens were used for AFM, which were also used for site-specific mechanical properties using AFM-based nanoindentation, and X-ray microprobe techniques. Thin-sectioned specimens (1–5 μm thickness) were prepared out of the remaining specimens and mounted on 100 nm thick silicon nitride (Si_3N_4) membranes for nano-TXM. It should be noted that specimens were taken from the root using the first two-thirds for acellular cementum and last one-third for secondary cementum as location indicators [14].

Deparaffinized sections for conventional histology and immunohistochemistry

Extracted molars (N = 5) containing PDL and alveolar bone were prepared for histology as stated in our previous work [6]. The sections were subsequently stained with hematoxylin and eosin (H&E) and picrosirius red (PSR). Stained tissues were characterized for structural PDL orientation and its integration with bone and cementum, using a polarized light microscope (BX 51, Olympus America Inc., San Diego, CA) to enhance the birefringence of collagen within alveolar bone and cementum. Images were acquired using Image Pro Plus v6.0 software (Media Cybernetics Inc., Silver Springs, MD).

Antibody tagging and localization

Antibody tagging was performed using the procedures established and described in our previous works [3]. In brief, small leucine rich proteins (SLRPs), biglycan (BGN), and fibromodulin (FMOD) within alveolar bone, periodontal ligament (PDL), PDL-bone and PDL-cementum entheses were identified.

Following deparaffinization of mounted sections with xylene, antigen retrieval was performed using trypsin digestion [3]. The following modifications to the manufacturer's protocol were made: FMOD, BGN stains, and respective antibodies from polyclonal rabbit sera were acquired from Dr. Larry Fisher (NICDR/NIH, Bethesda, MD). Specimens were blocked at room temperature for 20 min in 1% bovine serum albumin (Sigma, St. Louis, MO), and 1.5% mouse serum (Sigma, St. Louis, MO) in PBS. Antibody incubation was then performed overnight (18 h) at 4 °C, with the appropriate antibody diluted in blocking solution (1:50 for anti-BGN, 1:100 for anti-FMOD). Slides were washed 3 times the following day in PBS for 5 min each. Secondary antibody incubation of mouse anti-rabbit-IgG conjugated to HRP (Sigma, St. Louis, MO) diluted 1:100 in blocking solution, was performed at room temperature for 30 min, and then washed 3 times in PBS for 10 min each.

3,3′-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 of epitope locations. The specimens were then counterstained with Gill's III Hematoxylin (Sigma), dehydrated through serial solutions of 80% alcohol, 95% alcohol, 100% alcohol, and xylene, and mounted with Permount (Sigma). An Olympus BX51 light microscope was used for imaging with analyses using Image Pro software (Media Cybernetics Inc., Bethesda, MD).

AFM, AFM-based nanoindentation, and μ-XRF characterization

Light microscopy (BX51, Olympus America Inc., San Diego, CA) was used to image the surface of ultrasectioned block specimens [15] (N = 5), to identify bundle and lamellar bone in alveolar bone, and cementum of the tooth. Block specimens were characterized using an AFM, AFM-based nanoindentation, and microprobe for micro-XRF. Light micrograph image acquisition and analysis of lamellar bone (specifically interlamellae and lamellae regions), and bundle bone were conducted using Image Pro Plus v6.0 software (Media Cybernetics Inc., Silver Springs, MD).

AFM for structural analysis

Semi-qualitative data representative of bundle–lamellar (LB–BB) bone interface, collagen periodicity, hygroscopicity of inter-lamellae, and PDL-inserts within bundle bone were performed using contact mode AFM (Nanoscope III, Multimode; DI-Veeco Instruments Inc., Santa Barbara, CA) under dry and hydrated conditions [16]. AFM micrographs were analyzed with Nanoscope III version 5.12r3 software (Nanoscope III, Multimode; DI-Veeco Instruments Inc., Santa Barbara, CA).