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Strain-guided mineralization in the bone–PDL–cementum complex of a rat periodontium

Kathryn Grandfield [a,1], Ralf-Peter Herber [b,1], Ling Chen [a], Sabra Djomehri [a], Caleb Tam [a], Ji-Hyun Lee [a], Evan Brown [a], Wood R. Woolwine III [a], Don Curtis [a], Mark Ryder [c], Jim Schuck [d], Samuel Webb [e], William Landis [f], Sunita P. Ho [a,*]

[a] Division of Biomaterials and Bioengineering, Department of Preventive and Restorative Dental Sciences, University of California San Francisco, San Francisco, CA 94143, United States
[b] Division of Orthodontics, Department of Orofacial Sciences, University of California San Francisco, San Francisco, CA 94143, United States
[c] Division of Periodontics, Department of Orofacial Sciences, University of California San Francisco, San Francisco, CA 94143, United States
[d] Materials Science Division, The Molecular Foundry, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, United States
[e] Stanford Synchrotron Radiation Lightsource, SLAC National Accelerator Laboratory, Menlo Park, CA 94025, United States
[f] Department of Polymer Science, The University of Akron, OH 44325, United States

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ABSTRACT

Objective: The objective of this study was to investigate the effect of mechanical strain by mapping physicochemical properties at periodontal ligament (PDL)–bone and PDL–cementum attachment sites and within the tissues per se.
Design: Accentuated mechanical strain was induced by applying a unidirectional force of 0.06 N for 14 days on molars in a rat model. The associated changes in functional space between the tooth and bone, mineral forming and resorbing events at the PDL–bone and PDL–cementum attachment sites were identified by using micro-X-ray computed tomography (micro-XCT), atomic force microscopy (AFM), dynamic histomorphometry, Raman microspectroscopy, and AFM-based nanoindentation technique. Results from these analytical techniques were correlated with histochemical strains specific to low and high molecular weight GAGs, including biglycan, and osteoclast distribution through tartrate resistant acid phosphatase (TRAP) staining.
Results: Unique chemical and mechanical qualities including heterogeneous bony fingers with hygroscopic Sharpey's fibers contributing to a higher organic (amide III – 1240 cm^{-1}) to inorganic (phosphate – 960 cm^{-1}) ratio, with lower average elastic modulus of 8 GPa versus 12 GPa in unadapted regions were identified. Furthermore, an increased presence of elemental Zn in cement lines and mineralizing fronts of PDL–bone was observed. Adapted regions containing bony fingers exhibited woven bone-like architecture and these regions rich in biglycan (BGN) and bone sialoprotein (BSP) also contained high-molecular weight polysaccharides predominantly at the site of polarized bone growth.
Conclusions: From a fundamental science perspective the shift in local properties due to strain amplification at the soft–hard tissue attachment sites is governed by semiautonomous cellular events at the PDL–bone and PDL–cementum sites. Over time, these strain-mediated events can alter the physicochemical properties of tissues per se, and consequently the overall biomechanics of the bone–PDL–tooth complex. From a clinical perspective, the shifts in magnitude and duration of forces on the periodontal ligament can prompt a shift in physiologic mineral apposition in cementum and alveolar bone albeit of an adapted quality owing to the rapid mechanical translation of the tooth.

1. Introduction

The periodontal ligament (PDL) is vascularized and innervated softer structural component that assumes the role of regulating functional loads in the tooth–PDL–bone fibrous joint. While this joint is optimally engineered by nature to transfer loads and maintain tissues for function, it is also exposed to the inevitable parafunction, that is, exacerbated forces in the form of bruxism, jaw clenching, nail biting,

* Corresponding author at: Division of Biomaterials and Bioengineering, Department of Preventive and Restorative Dental Sciences, 707 Parnassus Avenue, University of California, San Francisco, CA 94143, United States.
E-mail address: sunita.ho@ucsf.edu (S.P. Ho).
[1] Both authors contributed equally.

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altered loads resulting from hardness variation in diet intake, and/or through clinical interventions such as the application of orthodontic appliances. The common denominator and a notable factor for the aforementioned conditions include eccentrically placed mechanical loads (Jang et al., 2014; Lin et al., 2013). Biomechanically, an optimal PDL-space is thought to permit the redistribution and transfer of loads from the tooth to alveolar bone specifically when loads are placed along the axis of the bone–tooth complex. This configuration constitutes a concentric loading system, which most often in nature prompts an optimum function when loaded within physiological limits. It is thought that the resulting optimum PDL-space enables force transfer across the mineralized tissues of this complex organ. Hence the PDL-space along with the PDL has also been suggested to act as a vital construct along which alveolar bone remodels/models (Beertsen et al., 1997), with the potential for cementum formation and resorption to also occur. Therefore, an improved knowledge of the “mechano-responsive” nature of the PDL, specifically at the PDL–bone and PDL–cementum attachment sites is relevant to understanding the strain-induced responses to our clinical interventions. Mechanical forces exert strains at the attachment sites and can “turn on” cell-surface receptors and consequently a cascade of biochemical signals, which in turn cause mineral formation and resorption related events in bone and/or cementum.

There exist many clinical interventions in skeletal (distraction osteogenesis), and oral and craniofacial orthopedics (for example, cranial grafts, orthodontics) that involve the use of mechanical forces to “mold” and/or regenerate bone and subsequently its adjacent tissues. However, very little is known about the influence of mechanical stimulus on the biomineralization of tissues per se within the bone–PDL–tooth complex or the functional interfaces between the ligament–bone and ligament–cementum. The current doctrine regarding the role of PDL in response to applied loads has been summarized as: 1) the PDL distributes applied loads to the alveolar bone, 2) the direction, frequency, duration and magnitude of loading determine both the extent, rate of bone remodeling and quality of modeled bone, 3) the absence of PDL severely limits the extent of bone remodeling (Beertsen et al., 1997). In this study, we propose that the mechanoresponsive activity at the PDL–bone and PDL–cementum attachment sites responds to eccentric loading by mineral formation and/or resorption at these sites by altering the functional space between the tooth and bone.

The importance of functional adaptations of the bone, in particular at interfaces, is not limited to orthodontics, but extends into the orthopedics. Within orthopedics, it has been suggested that functional adaptation of the bone results from cellular responses to strain density within a softer matrix (Carter et al., 1987). In this study, we identify the effects of perceived unidirectional forces on mineralization within the bone–PDL–tooth joint, and specifically at the soft–hard tissue interfaces of the PDL–bone and PDL–cementum sites and correlate it to the cellular and tissue adaptations in a rat model. The specific sites that were mechanically stimulated included the PDL–bone and the PDL–cementum functional attachment sites. Due to the nature of the ligament–bone interfaces in the musculoskeletal system, including the ligament–cementum interfaces in the bone–PDL–tooth complex, i.e. disparate tissues interfacing over a distance of 10 μm, these sites are mechanically strained (Lu and Thomopoulos, 2013; Qian et al., 2009) and the rate of adaptation at the interfaces is higher compared to other modeling sites. Furthermore, the attachment sites present themselves as excellent model systems where mechano-responsiveness, i.e. the response of mechanical strain amplification on mineral formation and resorption can be investigated and better understood. However, based on literature, mechano-responsiveness is assumed to occur at two different sites, the soft–hard tissue attachment site per se, and 5 μm inwards defined as an interface where the soft tissue transitions into a harder tissue.

Fundamentally, from a structural engineering perspective, eccentric loads are thought to accentuate strains, specifically at regions where dissimilar materials are attached. This concept was examined in this study, where strains at the functional interfaces and attachment sites of the PDL–bone and PDL–cementum were amplified by placing an eccentric load on the periodontium of a rat for fourteen days. In particular, cellular morphological phenotype and tissue adaptations were correlated to matrix structure, mechanical properties, and biochemical and elemental compositions facilitated through various techniques, including immunohistochemistry, microcomputed X-ray tomography (micro-XCT), microprobe X-ray fluorescence (micro-XRF), micro Raman spectroscopy and nanoindentation. The importance of this study lies in complementary higher-resolution imaging techniques combined with conventional histological and immunohistochemical measures allowing an understanding of the effect of mechanical strains at the interfaces of the bone–PDL–tooth complex. Thus, the objective of this study is to investigate gross morphological changes within tissues of the bone–PDL–cementum complex, changes in architecture and physicochemical characteristics of the formed structures due to amplified strain at the PDL–bone attachment site and interface.

2. Materials & methods

Please see appendix for details on the animal model used in this study (Fig. S1). In brief, 60 cN of force was applied to molars and incisors of 4 month old male Sprague–Dawley rats (Charles River Laboratories International Inc., Wilmington, MA, USA). Following 14 days of mechanical stimulus, the specimens (N = 16, 8 experimental and 8 in control group) were harvested and prepared for further characterization. The study was conducted according to the regulations of the animal protocol AN080608-02, approved by the Institutional Animal Care and Use Committee (IACUC), University of California, San Francisco (UCSF).

2.1. Micro X-ray computed tomography (Micro-XCT™)

Entire dissected and fixed maxillae from experimental animals (N = 8) and control animals (N = 8) were placed in 70% ethanol, and were imaged at 2× magnification at 90 kVp, a power of 6 W and a camera binning of 2 using a Micro XCT-200 (micro-XCT, Xradia Inc., Pleasanton, CA, USA). For imaging of bone forming areas, magnification was increased to 10× and 20×. Exposure times were adjusted to yield 25% of the original X-ray intensity passing through the specimen and arriving at the detector. Tomographies were reconstructed (XMReconstructor, Version 7.0.2817, Xradia Inc., Pleasanton, CA, USA) and post-processed using the Xradia 3D viewer and Avizo® Fire 7.1 for 3D segmentation (Visage Imaging Inc., Version 5.2.2, San Diego, CA, USA).

The tomograms were also used to calculate volumes including bone volume (BV) and canal volume (Ca.V), and tissue volume (TV) based on previous works (Carter et al., 2013; Cooper et al., 2006; Parfitt et al., 1987). To note, “tissue volume” is referred to as “total volume” in this work, and is a sum of the BV and CV.

2.2. Histology and histochemistry

The appliance was removed and the specimens were cut into blocks consisting of the molars, PDL, and alveolar bone, only. After scanning with micro-XCT, the hemimaxillae were decalcified with ethylenediaminetetraacetic acid (EDTA) solution for 3 weeks. The decalcified specimens were dehydrated with 80%, 95% and 100% Flex alcohol (Richard-Allan Scientific, Kalamazoo, MI) before embedding in paraffin (Tissue Prep-II, Fisher Scientific, Fair Lawn, NJ). The paraffin blocks were sagittally sectioned on a rotary microtome (Reichert-Jung Biocut, Vienna, Austria) using a disposable steel blade (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). Sections were deparaffinized with xylene and rehydrated through a descending ethanol series before further use. The stained tissues were