Published in final edited form as:

Biomaterials. 2010 September ; 31(25): 6635–6646. doi:10.1016/j.biomaterials.2010.05.024.

The biomechanical characteristics of the bone-periodontal ligament-cementum complex

Sunita P. Ho^{1,*}, Michael P. Kurylo¹, Tiffany Fong², Stephen Lee¹, Hanoch D. Wagner³, Mark Ryder⁴, and G. W. Marshall¹

¹ Division of Biomaterials and Bioengineering, Department of Preventive and Restorative Dental Sciences, University of California San Francisco, San Francisco, CA 94143

² Xradia Inc., 5052 Commercial Circle, Concord, CA, USA 94520

³ Department of Materials and Interfaces, The Weizmann Institute of Science, Rehovot 76100, Israel

⁴ Division of Periodontology, Department of Oromaxillary Sciences, University of California San Francisco, San Francisco, CA 94143

Abstract

The relative motion between the tooth and alveolar bone is facilitated by the soft-hard tissue interfaces which include periodontal ligament-bone (PDL-bone) and periodontal ligament-cementum (PDL-cementum). The soft-hard tissue interfaces are responsible for attachment and are critical to the overall biomechanical efficiency of the bone-tooth complex. In this study, the PDL-bone and PDL-cementum attachment sites in human molars were investigated to identify the structural orientation and integration of the PDL with bone and cementum. These attachment sites were characterized from a combined materials and mechanics perspective and were related to macro-scale function.

High resolution complimentary imaging techniques including atomic force microscopy, scanning electron microscopy and micro-scale X-ray computed tomography (Micro XCT™) illustrated two distinct orientations of PDL; circumferential-PDL (cir-PDL) and radial-PDL (rad-PDL). Within the PDL-space, the primary orientation of the ligament was radial (rad-PDL) as is well known. Interestingly, circumferential orientation of PDL continuous with rad-PDL was observed adjacent to alveolar bone and cementum. The integration of the cir-PDL was identified by 1 to 2 μ m diameter PDL-inserts or Sharpey's fibers in alveolar bone and cementum. Chemically and biochemically the cir-PDL adjacent to bone and cementum was identified by relatively higher carbon and lower calcium including the localization of small leucine rich proteins responsible for maintaining soft-hard tissue cohesion, stiffness and hygroscopic nature of PDL-bone and PDL-cementum attachment sites. The combined structural and chemical properties provided graded stiffness characteristics of PDL-bone (E_r range for PDL: 10 – 50 MPa; bone: 0.2 – 9.6 GPa) and PDL-cementum (E_r range for cementum: 1.1 – 8.3 GPa), which was related to the macro-scale function of the bone-tooth complex.

*To whom correspondence should be addressed: Sunita P. Ho, Ph.D., Division of Biomaterials and Bioengineering, Department of Preventive and Restorative Dental Sciences, D2252, 707 Parnassus Avenue, University of California San Francisco, San Francisco, CA 94143, Phone: 415-514-28183.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

Interfaces; Bone-Tooth Complex; Biomechanics; Fibrous Join Cementum; Alveolar Bone

1. INTRODUCTION

Stiffness graded interfaces within a multitude of natural systems provide a gradual change in mechanical properties from one dissimilar tissue to another, thus allowing function [1] while limiting high stress concentrations [2,3]. These well optimized interfaces are considered as biomimetic models to address some of the current challenges in tissue attachment [1]. In this study, attachment sites between hard and soft load bearing tissues that are vulnerable sites impacted by disease and/or traumatic loads [4,5] are sites of interest.

In a load bearing skeletal system several soft to hard tissue interfaces facilitate relative motion while resisting biomechanical loads. These include bone-tendon and bone-ligament attachment sites also termed as entheses [6]. At the enthesis, mechanical load is transferred from the compliant organic tissue such as tendon or ligament to a more rigid predominantly inorganic tissue such as bone. Mechanistically, these regions exhibit excellent load transmitting characteristics, however, from a biological perspective; they have higher levels of remodeling and modeling under mechanical loads [7,8]. Extraneous load induced perturbations or disease, enhance cellular activity and local inflammation often resulting in loss of joint function [9]. Subsequent long-term adaptation of these sites to external perturbations results in pathological disorders also known as enthesopathies that are common problems in the skeletal system [10].

In the dental system, the fibrous periodontal ligament [PDL] attaches cementum of the tooth to the alveolar bone [11]. Unlike the ligaments in the skeletal system, the bulk PDL contains neurovascular elements. This soft tissue between two mineralized bodies facilitates relative motion between the tooth and bone, and distributes cyclic masticatory forces [11]. These physiological forces are considered to be short-term and allow continuous adaptation of the bone-PDL-cementum complex [11,12]. However, the entheses in the dental system are also susceptible to degradation due to external perturbations such as traumatic or non-physiological loads as seen during tooth movement in orthodontic treatment or disease causing loss of function as in periodontal disease [13–15].

As adaptive changes in the bone-PDL-cementum complex results in changes in its structure and properties, this study sought to provide baseline information on the local structure, chemical composition and mechanical properties of PDL-bone and PDL-cementum entheses sites in healthy human molars. Furthermore, since structure regulates function, detailed structural integration of the softer PDL with the harder bone and cementum are examined using various complementary imaging modalities. This is to provide insight to the overall macro-scale biomechanical function relating to micro-scale adaptation of a bone-PDL-cementum complex that defines the tooth attachment apparatus. These investigations serve as a baseline to perform comparative studies on fundamental mechanisms responsible for adaptation of the periodontium under non-physiological inflammation leading to insights that provide more effective clinical interventions.

2. MATERIALS AND METHODS

The objectives of this study were twofold: 1). Investigate the structure, chemical composition and mechanical properties of healthy PDL-bone to PDL-cementum attachment sites; 2). Define macro- and micro-scale integration of PDL with bone and cementum in

healthy human molars. All teeth used in this study were periodontally healthy, and teeth extracted as a part of dental treatment were used in this study.

2.1. Ultrasectioned specimens for AFM, microindentation and AFM-based nanoindentation

Specimens containing molars ($N = 8$) and surrounding periodontal complex were obtained from 18 to 30 year old human subjects requiring extractions as a part of dental treatment following a protocol approved by the UCSF Committee on Human Research. Each specimen included the extracted molar, attached periodontal ligament and alveolar bone. The teeth were sterilized using 0.31 Mrad of γ -radiation [16]. Conventionally, primary cementum is defined as the first two-thirds of root length, and secondary cementum is defined as the last one-third of the root length i.e. the apical portion of a root [11].

The molars containing dentin, cementum, periodontal ligament, and alveolar bone were sectioned into $3 \times 3 \times 3$ mm cubes using a diamond wafering blade and a low-speed saw (Isomet, Buehler, Lake Bluff, IL) under wet conditions. The samples were glued to AFM steel stubs (Ted Pella, Inc., CA) using epoxy for ultra-sectioning with an ultramicrotome. A glass knife was employed to trim the blocks and a diamond knife (MicroStar Technologies, Huntsville, TX) was used to perform final sectioning by removing 300 nm thin sections [17]. The sectioned surface of the remaining block was characterized using an AFM and an AFM-based nanoindenter. Ultrasectioning of specimens' resulted in a relatively flat surface with low roughness permitting orthogonality between tip and specimen; a necessary criterion for AFM and indentation [17].

2.2. Deparaffinized sections for conventional histology and immunohistochemistry

Extracted molars ($N = 5$) containing PDL and alveolar bone were coarsely sectioned as detailed above. The sectioned specimens were fixed in sodium phosphate-buffered (pH 7.0) 4% formaldehyde for 3 days. The specimens were demineralized by immersing in Immunocal (Decal Chemical Corporation, Tallman, NY) formic acid solution [18] for eight weeks, with regular solution changes and agitation. The specimens were considered decalcified when addition of saturated ammonium oxalate to the solution failed to produce a precipitate.

2.2.1. Paraffin sections on microscope slides—The demineralized specimens were dehydrated with 80%, 95%, and 100% Flex alcohol (Richard-Allan Scientific, Kalamazoo, MI) and embedded in paraffin (Tissue Prep-II, Fisher Scientific, Fair Lawn, NJ). 5–6 μ m thick sections were achieved with a rotary microtome (Reichert-Jung Biocut, Vienna, Austria) using disposable steel blades (TBFTM Inc., Shur/SharpTM, Fisher Scientific, Fair Lawn, NJ). The paraffin serial sections were mounted on Superfrost Plus microscope slides (Fisher Scientific, Fair Lawn, NJ), deparaffinized with xylene then stained with Masson's trichrome. The stained tissues were characterized for structural orientation and integration of the PDL with bone and cementum, using a light microscope (BX 51, Olympus America Inc., San Diego, CA) and analyzed using Image Pro Plus v6.0 software (Media Cybernetics Inc., Silver Springs, MD). Polarized light was used to create a contrast between alveolar bone, PDL and the tooth.

2.2.2. Preparation of sections for antibody tagging—The mounted sections were deparaffinized in xylene, then rehydrated through serial solutions of 100%, 95%, and 80% alcohol. Endogenous peroxidases were deactivated by immersion in 80% methanol, 0.6% hydrogen peroxide for 20 minutes, followed by 3×5 minute washes in water and ending in phosphate-buffered saline (PBS). Antigen retrieval followed in two steps. First, trypsin digestion was performed for 10 minutes at room temperature in 0.1% trypsin, 0.1% CaCl_2 , 20 mM Tris-HCl pH 7.8, followed by rinsing with PBS for 5 minutes. Second,

glycosaminoglycan (GAG) removal was performed enzymatically in 35 mM Tris-HCl pH 7.4, 35 mM sodium acetate, 15 mU/mL chondroitinase ABC (Seikagaku Biobusiness Corporation, Tokyo, Japan), 3 mU/mL keratanase (Seikagaku Biobusiness Corporation, Tokyo, Japan) for 1 hour at 37°C in a humidified chamber. The following procedures were implemented to identify localization of small leucine rich proteins (SLRPs) such as asporin, biglycan (BGN), decorin (DCN) and fibromodulin (FMN) within PDL and at the bone-PDL and cementum-PDL attachment sites.

2.2.3. Antibody tagging to the digested sections—A primary goat anti-asperin polyclonal antibody (Abcam, Cambridge, MA) concentration of 1:25 was used with the VECTASTAIN ABC Kit, Goat IgG (Vector Labs, Burlingame, CA) for staining asporin. The following modifications to the manufacturer's protocol were made: the secondary rabbit anti-goat antibody incubation includes 1% BSA, and the subsequent washing steps use PBS with 0.1% Tween-20 added. For BGN, DCN, and FBN staining, the respective antibodies were obtained from Dr. Larry Fisher (NIDCR/NIH, Bethesda, MD), and are all polyclonal rabbit sera. Specimens were blocked at room temperature for 20 minutes in 1% bovine serum albumin (Sigma, St. Louis, MO), 1.5% mouse serum (Sigma) in PBS. Antibody incubation was then performed overnight (18 hours) at 4°C, with the appropriate antibody diluted in blocking solution (1:50 for anti-BGN, 1:100 for anti-DCN and anti-FMN). The next day, the slides were washed 3 times in PBS 5 minutes each. Secondary antibody incubation of mouse anti-rabbit-IgG conjugated to HRP (Sigma), diluted 1:100 in blocking solution, was performed at room temperature for 30 minutes, and then washed 3 times in PBS 10 minutes each.

2.2.4. Staining of antibody localization—3,3'-Diaminobenzidine (DAB) Enhanced Liquid Substrate System (Sigma, St. Louis, MO) was used per manufacturer's instructions with an incubation of 1 hour 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 to characterize the slides using Image Pro software (Media Cybernetics Inc., Bethesda, MD).

2.3. Scanning electron microscopy (SEM) of cryofractured and ultrasectioned specimens

Molars (N = 6) containing intact alveolar bone were coarsely sectioned in the longitudinal and transverse direction as detailed above. All specimens were fixed in 4% formaldehyde for 3 days followed by serial dehydration of 25%, 50%, 75%, and 95% ethanol for 30 minute each, and lastly washed with 100% ethanol for one hour. The specimens were immersed in liquid nitrogen for 5 minutes, followed by fracturing with a razor blade and a hammer.

The topography of the freshly fractured surfaces was characterized by mounting the specimens on SEM stubs followed by a 100 nm sputter coating of gold-palladium (Hummer VII, Anatech Ltd., VA, USA). The specimens were examined using an SEM (S4300, Hitachi, Tokyo, Japan) with an electron energy of 10–15 keV. In addition to performing electron microscopy on ultrasectioned specimens, energy dispersive X-ray (EDX) area mapping identifying carbon (C) K α 0.28 Kev and calcium (Ca) K α 3.69 Kev lines [19] within the circumferential-PDL (cir-PDL) and mineralized tissue was performed at the same electron energy.

2.4. Atomic force microscopy of ultrasectioned specimens

The ultrasectioned surfaced-blocks were imaged using a light microscope to provide a macro-scale site reference and understanding of each specimen before a detailed micro- and

nano-scale characterization was performed using an AFM. Qualitative analyses of the topography were performed using a contact mode AFM (Nanoscope III, Multimode; DI-Veeco Instruments Inc., Santa Barbara, CA) under both dry and wet conditions. The ultrasectioned surface was scanned using a Si_3N_4 tip attached to a “V shaped” cantilever with a nominal normal spring constant of 0.03 Nm^{-1} (DI-Veeco Instruments Inc., Santa Barbara, CA) at a scanning frequency of 1.4 Hz. The nominal radius of curvature of the tip was less than 50 nm. Scanning under wet conditions was performed with the specimen and probe immersed in deionized water as previously described [17]. Nanoscope III version 5.12r3 software (Nanoscope III, Multimode; DI-Veeco Instruments Inc., Santa Barbara, CA) was used for data processing.

2.5. Microhardness and AFM-based nanoindentation of ultrasectioned specimens

Micro-indentation on mineralized tissues using ultrasectioned blocks ($N = 8$) was performed under dry conditions with the use of a microindenter (Buehler Ltd., Lake Bluff, IL) and a Knoop diamond indenter (Buehler Ltd., Lake Bluff, IL). Each specimen was indented in dentin, cementum, alveolar bone, and entheses with each tissue containing approximately 5–20 indents. The distance between any two indents was chosen as per ASTM standard [20]. Microindentation was performed using a normal load of 10 gram force, and the long diagonal of each indent was immediately measured with the use of a light microscope with Image-Pro data-acquisition software (Media Cybernetics, Inc., Bethesda, MD).

The microhardness HK of respective regions was determined with the use of the following equation: $\text{HK} = 0.014229\text{P}/\text{D}^2$ where P is the normal load in Newtons (N) and D is the length of the long diagonal in millimeters (mm) [20]. The hardness values were determined by studying the residual impression of the microindents.

Wet nanoindentation ($N = 5$) was performed using an AFM, to which a load-displacement transducer (Triboscope, Hysitron Incorporated, Minneapolis, MN) was attached. A sharp diamond Berkovich indenter with a conventional radius of curvature less than 100 nm (Triboscope, Hysitron Incorporated, Minneapolis, MN) was fitted to the transducer. Site-specific measurements of reduced elastic modulus (E_r) and hardness (H) [21] were made under wet conditions using a displacement control of 500 nm penetration depth, with a load, hold, and unload for 3 seconds each. Fused silica was used to calibrate the transducer under dry and wet conditions. It should be noted that the direction of indentation load relative to fiber and/or lamella orientation can also influence the indentation modulus and hardness values.

2.7. Specimen preparation for micro X-ray computed tomography (Micro XCT™)

2 mm thick coarsely sectioned specimens were prepared from pre-fixed molars ($N = 5$) and were polished with 3 μm diamond slurry. Specimens containing intact cementum, PDL, and bone were fixed in sodium phosphate-buffered (pH 7.0) 4% formaldehyde for 5 days. Specimens were stained overnight with phosphotungstic acid (PTA) in a 30:70 ratio of 1% PTA (w/v) to 70% absolute ethanol [22]. All specimens were thoroughly rinsed, stored, and imaged in 50% ethanol with a tungsten anode setting at 40 keV, 8W using a 10X and a 20X magnifications (Micro XCT™, Xradia Inc., Concord, CA). Computed tomography (CT) was used to study the 3D structure of bone-PDL-cementum complex and allowed selection of virtual parallel slices spaced by 1 μm in different planes, thus illustrating the bulk structure of tissues. Specimens were enclosed in plastic wrap (Saran™, S. C. Johnson & Son, Inc., Racine, Wisconsin) to image under wet conditions.

3. RESULTS

In this study, the microstructure of complex interfaces between the soft tissue, PDL and hard tissues of cementum (C) and alveolar bone (AB) were investigated using a range of complementary techniques at a lower and higher resolution which included light, atomic force and electron microscopy techniques, and X-ray computed tomography. The microstructure was correlated to the chemical composition using immunohistochemistry and EDX analysis, and site specific mechanical properties using micro- and nano-indentation techniques.

3.1. Structural analysis of circumferential- and radial-fibrous PDL within the PDL-space

The Masson's trichrome staining (Fig. 1a) of 5 μm thick demineralized sections illustrates the collagen-rich PDL tissue (heavily stained blue), alveolar bone with several blood vessel spaces, cementum, and dentin. Under polarized light, the PDL inserts within alveolar bone are highlighted illustrating a woven fabric-like structure (Fig. 1b and 1c) similar to previously reported secondary cementum structure [23]. Additionally, figures 1b and 1c illustrate blood vessel spaces in alveolar bone and the PDL as perforations within the respective tissues. From these sections, it was noticed that the 100 – 200 μm wide radial-PDL (rad-PDL) spans the PDL space and changes its direction into a circumferential-PDL (cir-PDL) as it approaches the adjacent cementum and alveolar bone mineralized tissues (Figs. 1c and 1d). The cir-PDL fibers integrate with mineralized tissues via 1–2 μm wide collagen fibers, commonly known as Sharpey's fibers or PDL-inserts (dotted lines in Fig. 1d). At higher magnification (Fig. 1d) the presence of a consistent 5–10 μm organic-rich layer illustrating the enthesis region of PDL-bone and PDL-cementum can be seen (blue layer with asterisk marks). It should also be noted that the PDL between the two mineralized tissues is continuous with the fibrous tissue in the blood vessel spaces of the alveolar bone (Fig. 1a).

Masson's trichrome is not tissue specific, but does stain collagenous protein blue [18]. Hence, the PDL-specific SLRP known as asporin, was identified to complement the conventional histology results using immunohistochemistry. Figure 2 illustrates similar micro-anatomical locations within the tooth attachment apparatus of a human molar. Staining of the antibody can be observed within the rad-PDL and cir-PDL. Additionally, the staining of soft tissue within the blood vessel spaces of the alveolar bone confirms the continuity of the PDL from the commonly known PDL-space. However, asporin, considered to be a PDL specific SLRP, was not identified in predentin, or gingival tissue which are commonly used as internal positive controls (within the same histology section) to identify localization of other SRPs such as biglycan, decorin and fibromodulin (Fig. 2b – only biglycan is shown, however, similar localization was observed for decorin and fibromodulin).

To overcome processing artifacts of conventional histology, high resolution computerized tomography of 2 mm thick sections taken from human molars with PDL and alveolar bone was performed. Figure 3 illustrates the 3D structural arrangement of the bone-PDL-cementum complex. Tomographs (Figs. 3a and 3c) and corresponding 2D slices (Figs. 3b and 3d) along the wider plane of XZ illustrate the continuity between the cir-PDL, rad-PDL along with the blood vessel spaces, while Figures 3e and 3f illustrate the tomography of the thinner region and a thinner slice taken from plane YZ.

2D slices in figures 3b and 3d shows an intact vascularized PDL, various regions containing rad-PDL and cir-PDL complementing results obtained from conventional histology. In addition, the prevalence of circumferential tissue immediately lining the first ~ 20 μm of the cementum-PDL/AB-PDL interface can be detected. The change in direction of the PDL from rad-PDL to cir-PDL adjacent to the mineralized tissues might accommodate the blood

vessels within the rad-PDL that mostly lies within the PDL space. Additionally, depending on the size of the specimen thickness and the 2D slice, only cir-PDL or a combination of cir-PDL and rad-PDL were observed.

3.2. Integration of circumferential-PDL with alveolar bone and cementum

Ultrasectioning and cryofracturing of the coarsely sectioned specimens allowed high resolution imaging of the fibrous tissue integration using SEM and AFM including integration of collagen fibrils with the respective mineralized matrices. Both techniques illustrated rad-PDL and cir-PDL. The left and right columns in Figure 4 illustrate hierarchical length scale integration of the PDL tissue with bone and cementum. Figure 4a illustrates alveolar bone (AB), cementum (C), and PDL including PDL inserts within AB and cementum. The 1–3 μm wide radial PDL inserts, originate from the cir-PDL adjacent to respective bulk mineralized tissues (Figs. 4c, 4e and 4f).

Figure 4b illustrates site-specific radial PDL-inserts. In locations of the PDL containing vasculature, it should be noted that the 100–200 μm wide rad-PDL does not directly insert into cementum and alveolar bone as commonly understood. The course of direction of fibers within the 100–200 μm wide rad-PDL change to 10–50 μm wide cir-PDL (Figs. 4a, 4b) further splitting into 1–3 μm wide radial PDL-inserts (Figs. 4c, 4e and 4f) into respective mineralized tissues. The density of the radial PDL-inserts is different between cementum and alveolar bone as reported previously by others [11]. Higher resolution studies under wet conditions were performed on ultrasectioned blocks using an AFM. At a higher magnification the cir-PDL adjacent to cementum (same observations were made on the alveolar bone side – image not shown) along with the radial PDL-inserts can be seen in Figures 5b, 5c and 5e. Upon hydration, these regions exhibit partial swelling (lighter areas in Figure 5c). Furthermore, the intrinsic collagen periodicity in the cir-PDL can be observed; however, this periodicity is lost as the cir-PDL approaches respective mineralized tissues (Fig. 5d).

Energy dispersive X-ray (EDX) analysis was performed to complement AFM and immunohistochemistry results by demonstrating the presence of an organic-rich layer adjacent to bone and cementum. EDX of the cir-PDL integrating with bone and cementum (Fig. 6a) revealed a decrease in carbon (C) (Fig. 6b), an increase in calcium (Ca) (Figs. 6c) as expected.

3.3. Mechanical properties of the bone-PDL-cementum attachment apparatus

Micro- and nano-mechanical properties of the periodontium were collected based on Knoop hardness and AFM-based nanoindentation techniques. Care was taken to perform microindentation close to PDL-cementum and PDL-AB interfaces while avoiding indents which could be related to edge effects.

Using microindentation, Students' t-test with 95% confidence interval illustrated that PDL-C was significantly harder ($P < 0.05$) than PDL-AB enthesis sites. There was no significant difference ($P > 0.05$) between bulk cementum and PDL-C enthesis. However, the bulk alveolar bone was significantly harder than PDL-AB and PDL-C entheses sites (Table 1). As expected, tubular dentin was significantly harder ($P < 0.05$) than cementum, alveolar bone and their respective entheses sites with PDL (Table 1).

Although gross macro-scale differences were noticeable using microindentation under dry conditions, the modulus graded properties of the bone-PDL-cementum complex were illustrated using AFM-based nanoindentation under wet conditions. Additionally, site-specific properties of cementum-PDL and bone-PDL entheses sites, and bulk cementum and bone tissues, including the PDL under wet conditions were also evaluated. Owing to

limitations in specimen preparation and experimental technique including approximations in the classic Oliver-Pharr method [21] used for evaluating E_r , it should be noted that the evaluated mechanical properties are to establish relative comparisons between tissues and respective sites, and are not reported as absolute values. Furthermore, the rad-PDL characterized in this study was compromised due to specimen preparation and can only be taken as an approximate value [24]. Regardless, a representative gradient established from the cir-PDL and its association with the respective mineralized tissues is shown in Figure 7. Interestingly, the gradient representative of cir-PDL, cementum, cementum dentin junction (CDJ), and tubular dentin on the tooth side was more pronounced than the gradient of cir-PDL and alveolar bone on the side of the alveolar process. The E_r values evaluated using nanoindentation had a similar trend to microhardness values of respective regions (Table 1).

4. DISCUSSION

The goal of this study was to evaluate the bone-PDL-cementum complex of human molars from a combined materials and mechanics perspective. The first step was to identify the natural integration of the PDL with bone and cementum by evaluating the local structure, chemical composition and mechanical properties so that subsequent studies can relate macro-scale compressive loads to the micro-scale integration of the PDL with bone and cementum. Multiple complementary techniques were required to validate our findings accounting for specimen processing artifacts, with the most confirmatory evidence provided by microcomputed tomography. The significant challenge in this study was to preserve the delicate architecture of the PDL while understanding its integration with bone and cementum.

The integration between ligament and/or tendon, and bone in the musculoskeletal system, is classified into two types: the fibrocartilaginous enthesis and the fibrous enthesis [25]. The fibrocartilaginous enthesis allows large relative motion in diarthroidal joints contrary to limited motion in fibrous joints that are formed by fibrous enthesis. The fibrocartilaginous enthesis is thought to be chemically graded resulting in a well optimized load bearing interface. Histologically, the chemical composition varies from soft tissue to hard tissue with distinction of four well integrated regions that include the soft tissue such as a ligament or a tendon followed by uncalcified fibrocartilage, calcified fibrocartilage, and bone i.e. hard tissue. The less prevalent fibrous entheses found in fibrous joints known as gomphoses, which include the tooth-bone complex and sutures within the cranium [11], are characterized by the attachment sites in which there is direct insertion of the ligament into mineralized tissue. Regardless of the integration mechanism, the enthesis concept is well integrated in orthopedics. The results from this study indicate that the PDL attachment with alveolar bone and cementum forms a fibrous entheses.

The PDL-cementum and PDL-bone attachment sites are of particular interest since they are excellent biomimetic models for soft-hard tissue attachment. The mechanical properties of the PDL ligament under various compromised conditions have been reported [24], but to the authors knowledge no published work has addressed the structure, chemical, and mechanical aspects of the PDL-cementum and PDL-bone interface sites which are responsible for anchoring the ligament while dissipating stress caused by physiological and/or non-physiological loads. This study provides a necessary baseline for future studies which include investigating adaptation of the PDL-bone and PDL-cementum interfaces due to disease, age, excessive occlusal loads including traumatic and therapeutic loads due to orthodontic forces. Furthermore, from a tissue engineering perspective it is important to understand the integration of the load bearing dissimilar tissues i.e. soft tissue with the harder tissues to engineer biomechanically efficient replacements.

A significant challenge in this study was to preserve the structural integrity of the PDL such that the soft-hard tissue integration from a biomechanics perspective can be better understood. As a first step, the bone-PDL-cementum was demineralized and infiltrated with a polymer (paraffin) to facilitate sectioning followed by staining with Masson's trichrome to illustrate collagen and identify PDL-specific asporin [26] and other SLRP localizations using histochemistry. The challenge in this study was to keep PDL integration with bone and cementum intact. Based on tissue dissimilarity owing to differences in structure, chemical composition and mechanical properties, accompanied with harsh chemical processing, it was inevitable that structural integrity of the soft-hard tissue integration is compromised. This can be identified by structural relief caused in the thinner sections when removed from blocks compounded by chemical processing and gross sectioning artifacts. Despite the lack of dimensional accuracy in histological sections, the rad-PDL is continuous with cir-PDL within the PDL space (Fig. 1).

In order to minimize structural loss of PDL, PDL-bone, and PDL-cementum while maintaining a relatively flatter surface for dry and wet micro- and nano-scale characterization, ultramicrotomy was performed on undemineralized blocks of tooth-bone complex. In this study, the PDL soft tissue was not infiltrated and specimen sectioning was performed. It should be noted that drying of softer tissues causes collapse of the fibrillar structures and results in denser tissue (Fig. 4). Regardless, continuity between cir-PDL and rad-PDL fibers within the PDL space (Figs. 4a–4c) can be observed. The cryofracturing technique was used to illustrate the cir-PDL and its integration with adjacent mineralized tissues complementing the observations made using an AFM and SEM on ultrasectioned specimens. However, it should be noted that the use of an SEM regardless of the specimen preparation could compromise the structural integrity of soft tissues unlike the use of CryoSEM [27]. Additionally, SEM in general does not provide a 3D structural representation of the softer and harder tissue which was one of the main objectives of this study.

Although PDL integration with bone and cementum was clearly observed using AFM and SEM characterization on ultrasectioned and cryofractured specimens (Figs. 4 and 5), the limitation of both techniques involves significantly compromising the highly water retaining softer tissue during specimens preparation as mentioned previously. Hence, Micro XCTTM was used to investigate the cir-PDL and rad-PDL association relative to bone and cementum under wet conditions. Imaging while in water at relatively higher resolutions illustrated the two dominant orientations of the PDL including its association with the blood vessels within the PDL space and vascular spaces in bone. This continuity would explain the direct supply of nutrients, immune cells, chemoattractants, and various molecules from consumed food and drugs which are required for metabolic activities of PDL in health and disease [15]. Despite the commonly reported changes in PDL fiber orientation (from tooth cervix to the apex) within the PDL space, the structural orientation identified by the presence of cir-PDL is unique regardless of the anatomical location.

Periodontal ligament contains various types of collagen including type I, III, V, VI and XII [28,29]. The basic structural unit of a collagen fiber is a fibril which runs radially in the rad-PDL and circumferentially around the tooth and bone in cir-PDL. Owing to its molecular structure and arrangement, collagen in soft tissue is commonly characterized by its periodicity. However, this periodicity is obscured as the fibril mineralizes and is coated with mineral and extrafibrillar proteins [30]. Structural analysis using an AFM illustrated a loss in apparent collagen periodicity within the PDL approaching bone and cementum (Figure 4). Furthermore, mineralization of cir-PDL as it approaches bone or cementum can be seen by the dominance of calcium (Ca) using EDX.

At the soft-hard tissue attachment sites the transition from cir-PDL to respective mineralized tissues was identified by a 5–10 μm predominantly organic layers on respective mineralized tissues (Fig. 1). Biochemically, noncollagenous proteins (NCPs) such as osteopontin and osteocalcin which are thought to contribute toward regulation of mineralization and tissue cohesion have been identified at the attachment sites of the PDL-bone and PDL-cementum [29]. Other NCPs such as biglycan, fibromodulin and decorin were also identified at the PDL-bone and PDL-cementum attachment sites (Fig. 2).

In this study, the extent of PDL tissue integration with respective mineralized tissues was identified by determining the localization of the PDL-specific SLRP known as PLAP-1 or asporin [31,32] (Fig. 2). Similar to decorin and biglycan, asporin is a class I SLRP, but differs by not being a proteoglycan [32]. Additionally, asporin consistently was identified in the PDL and in dental follicle, the progenitor tissue that forms cementum, alveolar bone and the PDL. Owing to the load bearing characteristics of both cartilage and PDL, the role of asporin in collagen calcification has been associated with loss of joint mobility due to osteoarthritis and ankylosis [31–33]. Although asporin does not contain any glycosaminoglycans [26], the other commonly observed hydrophilic components, such as; chondroitin-sulfated and keratin-sulfated proteoglycans in biglycan, decorin and fibromodulin attract water molecules which in turn increase the water retention characteristics of the PDL-bone and PDL-cementum attachment sites (Fig. 5). It is known that the presence of hydrophilic molecules helps modulate cell migration and adhesion, tissue/interface mineralization, and other biochemical and biomechanical processes responsible for continuous remodeling of these biomechanically active sites e.g. the PDL-cementum and PDL-bone attachment sites. Therefore, any variation in macro-scale biomechanical load could alter the local strain levels in the PDL and at the PDL-bone and PDL-cementum attachment sites, leading to integrin based cell-matrix response and cell expressions causing detrimental or advantageous downstream effects [34]. The locally affected cellular expressions at the attachment sites could result in local changes in chemical composition including mineralization resulting in altered site-specific mechanical properties as a local tissue adaptation to macro-scale loading.

Despite the optimum load bearing function of the soft-hard tissue attachment sites, they exhibit higher level of remodeling [7] due to the transmission of mechanical load from a compliant organic tissue to a more rigid predominantly inorganic tissue. For the same reason, in the musculoskeletal system, these biomechanically active sites are considered vulnerable due to various enthesopathies i.e. pathological disorders at the soft-hard tissue attachment [10]. However, correlating these observations with classical engineering theory on interfaces [3], the degree of the pathological disorder at the attachment site could vary depending on the magnitude of the gradient. A higher gradient implies a sudden increase in elastic modulus over a narrower interface, which could lead to a rapid rate of soft-hard attachment site degeneration when compared to a lower gradient where a gradual increase in elastic modulus from a soft to a hard tissue [3,35] over a wider interface is advantageous.

Under normal conditions, the graded stiffness from PDL to bone and PDL to cementum could vary due to 1) the natural physiological movement of teeth with age known as active eruption [36], and 2) biomechanical function; masticatory forces due to normal and malocclusion [15]. This normal adaptive role of the PDL-bone and PDL-cementum interfaces is amplified with the addition of external perturbations such as therapeutic load caused by orthodontic forces or disease as is the case with periodontitis [13–15,34]. In some cases, while adaptation is advantageous, in certain cases it could compromise the overall biomechanical efficiency of the tooth-bone complex. Furthermore, other influencing factors include age related changes in soft and hard tissues. Hence age should be considered when accounting for studies on structure-function relationships of load bearing organs.

In this study, the partial swelling at the hypomineralized regions within the attachment sites between PDL and bone or cementum could establish the interface width over which the mechanical properties vary from the lower PDL (10 – 50 MPa) properties to the higher values observed in alveolar bone (0.2 – 9.6 GPa) and cementum (1.1 – 8.3 GPa) (Fig. 7, Table 1). Much like the interfaces between dentin and enamel, and cementum and dentin within a tooth, the functional interface between PDL and cementum, and PDL and bone is graded in elastic modulus and hardness (Fig. 7). The graded elastic modulus and hardness provides optimum transfer of loads between the dissimilar materials as indicated by the significant differences in hardness using microindentation (Table 1). The gradation in properties on the PDL-bone side could be contributed by the cir-PDL (Fig. 1a) and osteoid layer which is adjacent to lamellar bone [37]. On the PDL-cementum side, the gradation in properties is contributed by cir-PDL adjacent to a hypomineralized cementum-like layer (Fig. 1d); pre-cementum [38], followed by bulk cementum interfacing with root dentin. It should be noted that alveolar bone and cementum are heterogeneous tissues which is the reason why ranges of elastic modulus and hardness were reported (Table 1). However, in case of microindentation, because the indenter was 1000 times larger, the heterogeneity of materials is masked providing an overall hardness value for each material. The observed heterogeneity in bone (Table 1) using nanoindentation is due to the various forms of bone caused due to tissue adaptation [36,37,39]. These include bundle bone adjacent to the lamellar bone. Additionally, the PDL-inserts in bundle bone, including the rich organic and inorganic lamellae in the lamellar bone similar to skeletal bone [40] could contribute to the observed range. The structure of cementum is similar to alveolar bone and includes a region rich in PDL-inserts, followed by lamellar cementum [41].

Macro-scale structure-function behavior at the organ level provides an insight to local behavior at the tissue level and resulting cellular responses and subsequent molecular expressions [42]. Based on fundamental biomechanics, the tooth is subjected to a variety of loads of which compressive loads dominate the occlusal surface (Fig. 8a). These functional loads are accommodated by the bulk properties of various mineralized tissue, PDL and the soft-hard tissue binding interfaces facilitating micro-motion between the tooth and the alveolar bone. Furthermore, the natural morphology of a tooth can cause tilting within the alveolar socket due to functional loads [43,44] leading to local changes in hydrostatic and distortional stress states. Considering only bone-PDL-cementum distortion and focusing first on region A (Fig. 8b), under small displacements the compressive occlusal loads result in pure shear stress state across the PDL space; however under larger displacements the PDL tissue undergoes progressively increasing shear and tension, supplemented by flexural moments at the tethered ends of the ligament with bone and cementum. We hypothesize that these bending moments result in high local stress concentrations and may thus play a role as a stimulant for cell activity and tissue remodeling as these moments lead to pulling forces that induce biological activity. Region B (Fig. 8b) behaves in a similar fashion in terms of stress distribution with slightly lower tensile stresses in the ligaments. As we move into region C (Fig. 8b) the stress profile becomes almost exclusively compressive, and tension as well as bending moments at the tethered ends could vanish. Hence, it is conceivable that degree of remodeling due to mechanotransduction is also dependent on the attachment/integration of the softer PDL with the harder cementum and bone. This would explain the higher concentration of the NCPs observed at the PDL-cementum and PDL-bone attachment sites (Fig. 2).

Correlating the structure, chemical composition and mechanical properties of the PDL integration with bone and cementum to macro-scale biomechanics was done by comparing the proposed bone-PDL-cementum model (Fig. 8d) with the current standard model which presents direct-bridging of PDL (Fig. 8c). Fundamentally, the observed properties could influence the following two conditions: 1) anchoring performance under tension and

compression of the bone-PDL-cementum complex (Fig. 8b), and 2) subsequent stress concentrations at the PDL-bone and PDL-cementum attachment sites (Figs. 8c and 8d). Regarding 1) comparing model 8c and 8d while keeping the total bridging volume a constant, the ratio of interfacial area to volume of the bridges is much larger in model 8d which is due to the presence of the cir-PDL adjacent to bone and cementum. This generates a more efficient interfacial stress transfer from the ligaments to the adjacent mineralized tissues. Regarding 2), the presence of cir-PDL leads to lower bending stress concentrations at the attachment sites of the PDL-bone and PDL-cementum, compared to direct bridging presented in 8c. Furthermore, the presence of cir-PDL allows stresses at the attachment sites to distribute along the direction of the fibers in cir-PDL first before dissipating into adjacent bone and cementum. The observed structural orientation and integration of the PDL including variations in chemical composition could elucidate the resulting mechanical properties and graded stiffness characteristics from bone, PDL to cementum.

5. CONCLUSIONS

The periodontal ligament is integrated with bone and cementum in both circumferential (cir-PDL) and radial (rad-PDL) directions. The transition from a softer tissue to a mineralized tissue is via graded interfaces, where the reduced elastic modulus increases gradually from PDL to bone and PDL to cementum within the bone-PDL-cementum complex. The structural integration along with the graded properties could explain the biomechanical efficiency of the bone-PDL-cementum complex. Additionally, this research work provides insight into the cause for local remodeling and resorption of the bone-PDL and cementum-PDL attachment sites; adaptation sites due to mechanotransduction.

Acknowledgments

The authors thank Prof. Stephen Weiner, Weizmann Institute of Science, Israel, and Prof. Ron Shahar, The Hebrew University of Jerusalem, Israel for many valuable technical discussions. Additionally, the authors thank Peter Sargent, Ph.D., Department of Cell and Tissue Biology, UCSF, for the use of the ultramicrotome, Lawrence Berkeley National Laboratory for the use of Scanning Electron Microscope and Linda Prentice at UCSF for histology. Support was provided by NIH/NIDCR R00 DE018212.

References

1. Fratzl P. Biomimetic materials research: what can we really learn from nature's structural materials? *J R Soc Interface* 2007;4:637–42. [PubMed: 17341452]
2. Genin MG, Kent A, Birman V, Wopenka B, Pasteris JD, Marquez PJ, et al. Functional grading of mineral and collagen in the attachment of tendon to bone. *Biophys J* 2009;97(4):976–85. [PubMed: 19686644]
3. Suresh S. Graded materials for resistance to contact deformation and damage. *Science* 292(5526): 244–51.
4. Benjamin M, Toumi H, Ralphs JR, Bydder G, Best TM, Milz S. Where tendons and ligaments meet bone: attachment sites ('enthese') in relation to exercise and/or mechanical load. *J Anat* 2006;208(4):471–90. [PubMed: 16637873]
5. Benjamin M, Moriggl B, Brenner E, Emery P, McGonagle D, Redman S. The "enthesis organ" concept: why enthesopathies may not present as focal insertional disorders. *Arthritis Rheum* 2004;50(10):3306–13. [PubMed: 15476254]
6. Benjamin M, Kumai T, Milz S, Boszczyk BM, Boszczyk AA, Ralphs JR. The skeletal attachment of tendons--tendon "enthese". *Comp Biochem Physiol A Mol Integr Physiol* 2002;133(4):931–45. [PubMed: 12485684]
7. Benjamin M, McGonagle D. The anatomical basis for disease localisation in seronegative spondyloarthritis at entheses and related sites. *J Anat* 2001;199(5):503–26. [PubMed: 11760883]

8. Roberts WE, Huja SS, Roberts JA. Bone modeling: biomechanics, molecular mechanisms, and clinical perspectives. *Semin Orthod* 2004;10(2):123–61.
9. Benjamin M, McGonagle D. Basic concepts of enthesis biology and immunology. *J Rheumatol Suppl* 2009;83:12–3. [PubMed: 19661528]
10. Claudepierre P, Voisin MC. The entheses: histology, pathology, and pathophysiology. *Joint Bone Spine* 2005;72:32–7. [PubMed: 15681245]
11. Nanci, A. Ten Cate's Oral Histology. Mosby; 2007.
12. Wagle N, Do NN, Yu J, Borke JL. Fractal analysis of the PDL-bone interface and implications for orthodontic tooth movement. *Am J Orthod Dentofacial Orthop* 2005;127(6):655–61. [PubMed: 15953889]
13. Henneman S, Von den Hoff JW, Maltha JC. Mechanobiology of tooth movement. *Eur J Orthod* 2008;30:299–306. [PubMed: 18540017]
14. Bartold PM, Narayanan AS. Molecular and cell biology of healthy and diseased periodontal tissues. *Periodontol 2000* 2006;40:29–49. [PubMed: 16398684]
15. Krishnan V, Davidovitch Z. On a path to unfolding biological mechanisms of orthodontic tooth movement. *J Dent Res* 2009;88(7):597–608. [PubMed: 19641146]
16. Brauer DS, Saeki K, Hilton JF, Marshall GW, Marshall SJ. Effect of sterilization by gamma radiation on nano-mechanical properties of teeth. *Dent Mater* 2008;24(8):1137–40. [PubMed: 18436298]
17. Ho SP, Goodis H, Balooch M, Nonomura G, Marshall SJ, Marshall G. The effect of sample preparation technique on determination of structure and nanomechanical properties of human cementum hard tissue. *Biomaterials* 2004;25(19):4847–57. [PubMed: 15120532]
18. Shehan, DC. Theory and practice of histotechnology. 2. The C. V. Mosby Company; 1980.
19. Thompson, A.; Attwood, D.; Gullikson, E.; Howells, M.; Je Kim, K.; Kirz, J., et al. X-ray Data Booklet. Thompson, AC.; Vaughan, D., editors. Lawrence Berkeley National Laboratory; 2001.
20. ASTM. E 384–99: Standard test method for microindentation hardness of materials. West Conshohocken, PA: American Standard for Testing Materials International;
21. Pharr GM, Oliver WC, Brotzen FR. On the generality of the relationship among contact stiffness, contact area, and elastic-modulus during indentation. *J Mater Res* 1992;7(3):613–7.
22. Metscher BD. MicroCT for developmental biology: a versatile tool for high-contrast 3D imaging at histological resolutions. *Dev Dyn* 2009;238(3):632–40. [PubMed: 19235724]
23. Ho SP, Yu B, Yun W, Marshall GW, Ryder MI, Marshall SJ. Structure, chemical composition and mechanical properties of human and rat cementum and its interface with root dentin. *Acta Biomater* 2009;5(2):707–18. [PubMed: 18829402]
24. Cattaneo PM, Dalstra M, Melsen B. Strains in periodontal ligament and alveolar bone associated with orthodontic tooth movement analyzed by finite element. *Orthod Craniofac Res* 2009;12(2):120–8. [PubMed: 19419455]
25. Benjamin M, McGonagle D. Entheses: tendon and ligament attachment sites. *Scand J Med Sci Sports* 2009;19(4):520–7. [PubMed: 19522749]
26. Yamada S, Murakami S, Matoba R, Ozawa Y, Yokokoji T, Nakahira Y, et al. Expression profile of active genes in human periodontal ligament and isolation of PLAP-1, a novel SLRP family gene. *Gene* 2001;275(2):279–86. [PubMed: 11587855]
27. Terada N, Ohno N, Li Z, Fujii Y, Baba T, Ohno S. Application of in vivo cryotechnique to the examination of cells and tissues in living animal organs. *Histol Histopathol* 2006;21(3):265–72. [PubMed: 16372248]
28. Berkovitz BK. The structure of the periodontal ligament: an update. *Eur J Orthod* 1990;12(1):51–76. [PubMed: 2180728]
29. McCulloch CAG, Lekic P, McKee MD. Role of physical forces in regulating the form and function of the periodontal ligament. *Periodontol 2000*;24:56–72.
30. Balooch M, Habelitz S, Kinney JH, Marshall SJ, Marshall GW. Mechanical properties of mineralized collagen fibrils as influenced by demineralization. *J Struct Biol* 2008;162(3):404–10. [PubMed: 18467127]

31. Yamada S, Tomoeda M, Ozawa Y, Yoneda S, Terashima Y, Ikezawa K, et al. PLAP-1/asporin, a novel negative regulator of periodontal ligament mineralization. *J Biol Chem* 2007;282(32):23070–80. [PubMed: 17522060]
32. Kalamajski S, Aspberg A, Lindblom K, Heinegard D, Oldberg A. Asporin competes with decorin for collagen binding, binds calcium and promotes osteoblast collagen mineralization. *Biochem J* 2009;423(1):53–9. [PubMed: 19589127]
33. Kizawa H, Kou I, Iida A, Sudo A, Miyamoto Y, Fukuda A, et al. An aspartic acid repeat polymorphism in asporin inhibits chondrogenesis and increases susceptibility to osteoarthritis. *Nat Genet* 2005;37(2):138–44. [PubMed: 15640800]
34. Krishnan V, Davidovitch Z. Cellular, molecular, and tissue-level reactions to orthodontic force. *Am J Orthod Dentofacial Orthop* 2006;129(4):469, e1–32. [PubMed: 16627171]
35. Ho SP, Joseph PF, Drews MJ, Boland T, LaBerge M. Experimental and numerical modeling of variable friction between nanoregions in conventional and crosslinked UHMWPE. *J Biomech* 2004;126(1):111–9.
36. Weinmann JP. Bone changes related to eruption of the teeth. *Angle Orthod* 1941;11(2):83.
37. Lindhe, J.; Karring, T.; Lang, NP., editors. *Clinical Periodontology and Implant Dentistry*. 2003.
38. Matthiessen ME, Sogaard-Pedersen B, Romert P. Electron microscopic demonstration of non-mineralized and hypomineralized areas in dentin and cementum by silver methenamine staining of collagen. *Scand J Dent Res* 1985;93(5):385–95. [PubMed: 3864211]
39. Weinmann JP. Variations in the structures of bone and bones and their significance in radiology. *Oral Surg Oral Med Oral Pathol* 1955;8(9):988–92. [PubMed: 13254127]
40. Fan Z, Swadener JG, Rho JY, Roy ME, Pharr GM. Anisotropic properties of human tibial cortical bone as measured by nanoindentation. *J Orthop Res* 2002;20(4):806–10. [PubMed: 12168671]
41. Ho SP, Marshall SJ, Ryder MI, Marshall GW. The tooth attachment mechanism defined by structure, chemical composition and mechanical properties of collagen fibers in the periodontium. *Biomaterials* 2007;28(35):5238–45. [PubMed: 17870156]
42. Carter, DR.; Beaupre, GS. *Skeletal function and form*. Cambridge University Press; 2001.
43. Qian L, Todo M, Morita Y, Matsushita Y, Koyano K. Deformation analysis of the periodontium considering the viscoelasticity of the periodontal ligament. *Dent Mater* 2009;25(10):1285–92. [PubMed: 19560807]
44. Chattah NL. Design strategy of minipig molars using electronic speckle pattern interferometry: comparison of deformation under load between the tooth-mandible complex and the isolated tooth. *Adv Mater* 2008;20:1–6.

Figure 1.

(a) Polarized light microscopy of a Masson's trichrome stained section illustrating alveolar bone (AB), periodontal ligament (PDL), cementum and dentin at a lower resolution. (b, c) Higher resolution micrographs illustrate bone containing radial fibers and circumferential fibers (double headed arrows). (d) Higher resolution micrograph illustrating cir-PDL and rad-PDL (double-headed arrows) in addition to a 5 μm thin collagenous tissue circumferential to bone and cementum (asterisks). The dashed arrows illustrate PDL-inserts in AB.

Figure 2.

(a, b) Light microscope micrographs of bone-PDL-cementum complex in human periodontium illustrating localization of asporin and biglycan within PDL-space. The localization of biglycan can be seen at the PDL-bone and PDL-cementum attachment sites (asterisks). (c) Representative light micrographs of a negative control (the micrographs for all SLRPs were similar).

Figure 3. Micro XCT™ of a bone-PDL-tooth complex illustrating the PDL integration with bone and tooth. (a, c). The 3D image illustrates the network of the cir-PDL and rad-PDL within the PDL space. The cir-PDL is adjacent to cementum and bone, and the rad-PDL is continuous with the cir-PDL. (b,d,f). Virtual histology sections taken from the 3D images illustrate the 2D network of the bone-PDL-cementum complex. (e, f). The thickness of cir-PDL compared to rad-PDL can vary depending on the sectioning plane.

Figure 4.

SEM micrographs of ultrasectioned (a-c) and cryofractured (d-f) specimens illustrating alveolar bone (AB), cir-PDL, rad-PDL and cementum. (a, b). The cir-PDL and rad-PDL can be observed at this resolution. Additionally, the vascular spaces (black voids) and continuity between the radial and circumferential fibrous PDL (double-headed arrows) can be seen. (c). Illustrates the mechanism of integration via a 2 μm wide PDL collagen fiber-insert with alveolar bone. Similar PDL-cementum integration was observed. (d). Notice the fibrous tissue adjacent to alveolar bone (white arrows in inset). (d-f). cir-PDL peeled from the bulk mineralized tissue under SEM vacuum conditions, revealing its integration with bone (white arrows) and cementum (black arrows) through ligament-like attachments (red asterisks) forming PDL-cementum and PDL-bone entheses sites.

Figure 5.

(a). Light microscopy image of an ultrasectioned block illustrating cementum, alveolar bone and the PDL space. High resolution AFM micrographs of cir-PDL (double headed black arrows) adjacent to the mineralized tissues are shown at different magnifications in figures b–e. (b, c). AFM of the fibrous tissue adjacent to cementum under dry (b) and wet (c) conditions. Notice the radial PDL-inserts in cementum forming the PDL-cementum entheses. Furthermore, the swelling of the collagen fibers within cementum can be observed (c). (d). At a higher resolution the circumferentially oriented fibers illustrate several collagen fibrils (double-headed arrows). Loss of collagen periodicity as the PDL approaches the mineralized tissue can also be observed. (e) AFM of dry bone illustrating PDL-inserts.

Figure 6. Energy dispersive X-ray mapping of the cir-PDL adjacent to cementum (a) illustrates relatively higher carbon levels in cir-PDL (b) in comparison to calcium (c) in cementum.

Figure 7.

Line profiles of reduced elastic modulus and hardness values for wet bone-PDL-cementum complex, illustrating a dominant gradient on the tooth side relative to bone.

Figure 8.

Schematic illustrating the integration of PDL with bone and cementum. (a). Macro-scale tooth-PDL-bone. (b). Schematic to explain stress states in regions A-C within the bone-PDL-cementum complex. (c). Micro-scale representation of bone-PDL-cementum architecture illustrating direct bridging of 100–200 μm wide collagen fiber bundles also known as PDL from bone to cementum. The collagen fiber bundle splits into several 1–2 μm collagen fibers within bone and cementum causing further integration. (d). Architecture of the bone-PDL-cementum complex derived from results presented in this study. The cir-PDL and rad-PDL are continuous and integrate with the mineralized tissues as shown by double-headed arrows. The integration is provided by splitting of the collagen fiber bundles into finer 1–2 μm collagen fibers within respective mineralized tissues. Relative to the bone, the tooth is considered to go through a whole body movement initially as shown by the blue arrow before bone and tooth deformation is observed. Please see discussion for further explanation of this figure.

Reduced Elastic Modulus and Hardness values using Micro and Nanoindentation
Microindentation data illustrating average hardness values of tissues and respective attachment sites. The asterisks indicate significant differences in hardness values of PDL-alveolar bone (PDL-AB), alveolar bone (AB), and tubular dentin relative to cementum and PDL-cementum (PDL-C) entheses. Table also includes ranges of reduced elastic modulus and hardness values using nanoindentation technique under wet conditions. These values are representative of heterogeneity of respective mineralized tissues and their attachment sites within the bone-PDL-cementum complex.

Table 1

Micro/Nanoindentation	Reduced Elastic Modulus 'E _r ' (GPa)					Hardness 'H' (GPa)				
	Bone	PDL-AB	PDL-C	Cementum	Tubular Dentin	Bone	PDL-AB	PDL-C	Cementum	Tubular Dentin
Microindentation (DRY)						0.56 ± 0.1 *	0.42 ± 0.1 *	0.5 ± 0.2	0.5 ± 0.1	0.64 ± 0.1 *
Nanoindentation (WET)	0.2–9.6	0.1–1.0	0.1–0.6	1.1–8.3	10–25	0.01–0.15	0.01–0.03	0.01–0.02	0.014–0.19	0.3–0.9

* Significant difference (P < 0.05) with respect to PDL-C entheses site and cementum