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Periodontal Ligament Entheses and their Adaptive Role in the Context of Dentoalveolar Joint Function

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Abstract

Objectives—The dynamic bone-periodontal ligament (PDL)-tooth fibrous joint consists of two adaptive functionally graded interfaces (FGI), the PDL-bone and PDL-cementum that respond to mechanical strain transmitted during mastication. In general, from a materials and mechanics perspective, FGI prevent catastrophic failure during prolonged cyclic loading. This review is a discourse of results gathered from literature to illustrate the dynamic adaptive nature of the fibrous joint in response to physiologic and pathologic simulated functions, and experimental tooth movement.

Methods—Historically, studies have investigated soft to hard tissue transitions through analytical techniques that provided insights into structural, biochemical, and mechanical characterization methods. Experimental approaches included two dimensional to three dimensional advanced *in situ* imaging and analytical techniques. These techniques allowed mapping and correlation of deformations to physicochemical and mechanobiological changes within volumes of the complex subjected to concentric and eccentric loading regimes respectively.

Results—Tooth movement is facilitated by mechanobiological activity at the interfaces of the fibrous joint and generates elastic discontinuities at these interfaces in response to eccentric loading. Both concentric and eccentric loads mediated cellular responses to strains, and prompted self-regulating mineral forming and resorbing zones that in turn altered the functional space of the joint.

Significance—A multiscale biomechanics and mechanobiology approach is important for correlating joint function to tissue-level strain-adaptive properties with overall effects on joint form as related to physiologic and pathologic functions. Elucidating the shift in localization of biomolecules specifically at interfaces during development, function, and therapeutic loading of

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the joint is critical for developing "functional regeneration and adaptation" strategies with an emphasis on restoring physiologic joint function.

I. Bone-Periodontal Ligament-Tooth Fibrous Joint

The bone-periodontal ligament (PDL)-tooth complex of the oral and craniofacial masticatory complex is a dynamic and biomechanically active fibrous joint (1). The primary function of the joint is to sustain cyclic chewing forces of varying magnitudes and frequencies. It is categorized as a fibrous joint by virtue of the PDL that 1) serves to attach teeth to the alveolar bone (1), a type of bone that is distinctly different from skeletal bone; 2) serves to consequently facilitate tooth displacement within the alveolar bony socket; 3) serves to distribute and dampen masticatory forces through the vascularized and innervated PDL; 4) contains differentiating zones at the ligament-cementum and ligament-bone entheses (attachment sites) (2, 3); 5) adjoins and interacts with cementum and alveolar bone through ligament-cementum and ligament-bone interfaces; 6) sustains and permits load-based reactionary forces from the tissues (enamel, dentin, cementum) and interfaces (enamel-dentin and cementum-dentin junctions) that makeup teeth exclusively; and 7) subsequently induce mechanical strain not limited to alveolar bone (4, 5). A multitude of structural components and tissues (categorized as biomaterials) join to form this nature's well-lubricated load-bearing joint. Conceivably, this fibrous joint undergoes mechanical strain-mediated adaptation where the measured physical and chemical properties of load-bearing tissues per se and their interfaces are appreciated in the context of overall function. Understanding how tooth motion is guided within the alveolar socket in the presence of the PDL, and how the attachment process accommodates cyclic functional loads is critical as it would allow extracting strain-adaptive information that promotes tissue regeneration/remodeling to maintain biomechanical function of a joint.

To date, the biomechanical aspects of the bone-PDL-tooth fibrous joint have been investigated at discrete length-scales, that is, at the levels of the joint (6–13), tissues (3, 14–18), and cells (19–29). Investigations at a joint level have provided insights into the "coupled" nature of joint form and its masticatory function (11). At a tissue level, biological processes identified as interactions between the soft organic meshwork of the PDL and adjoining hard bone and cementum matrices, and subsequent strain-mediated mineralization of the inorganic hard tissue through active modeling and remodeling (30, 31), have provided insights into joint adaptation in response to physiologic and pathologic forces (2, 8, 9, 31–34). Through a reductionist approach, at a tissue and cellular-level, immunohistological approaches led to mapping of cell behavior and related matrix protein expressions to perturbations placed on tissues and cultured scaffolds (2, 3, 18, 26, 28, 32, 34–36). This also implies that cell and related tissue mechanics are seldom evaluated within the context of organ function. While probing using reductionist approach answers questions specific to tissues and cells respectively, it minimally addresses the importance of the measured physical and chemical properties, and thereby biological processes within the realm of function. In this manuscript, insights into adapted features within a complex in the context of function will be extracted using principles from biomechanics and mechanobiology. As a result, the manuscript will also highlight an interdisciplinary yet holistic approach (as opposed to reductionist) through the use of various imaging modalities to detail the effect of form and function relationship on strain adaptive properties of human alveolar bone, the PDL, cementum, and PDL-bone and PDL-cementum interfaces. Additionally, results will be discussed in the context of clinically relevant problems specifically related to orthodontics.

The two central objectives to highlight the critical importance of regenerative capacity of PDL-bone and PDL-cementum interfaces within the context of multi-scale biomechanics of the bone-PDL-tooth fibrous joint will be as follows:

1. The changes in form of a human tooth relative to the socket-shape and vice versa can prompt local adaptations through strain concentrations within the PDL, PDL-bone and PDL-cementum interfaces that facilitate the dynamic function of the bone-PDL-tooth fibrous joint (8, 31). Within this objective the following salient points will be discussed.

    a The structural integration in tandem with the gradual variation in physical and chemical characteristics at the PDL-bone and PDL-cementum interfaces exist within a bone-PDL-cementum complex (32).

    b Deviation from normal PDL-space (150–380 μm) to adapted regions of (5–50 μm) (33) at the original ligament-bone and ligament-cementum attachment are to resist functional demands.

    c Over time, these constriction sites within the PDL-space are new load-bearing sites and could eventually impair joint function (31).

2. *In vivo* models can be used to manipulate and help understand the adaptive capacities of entheses/interfaces. “Natural intelligence” of interfaces can be determined by extracting mechanoresponsive signals from cells within local regions specifically within the context of a clinical setting such as orthodontic tooth movement (OTM) (37, 38).

    d The semiautonomous events at interfaces guide the joint within physiologic limits, but significant mineral formation and resorption at the PDL-interfaces may result in closing or widening of the PDL-space and can guide the complex to a nonphysiologic regime (9, 38).

In all cases, insights into the functional information for the PDL-bone and PDL-cementum interfaces will be extracted from *in situ* experiments (6, 39), and mapping of physical and chemical characteristics of interfaces will be performed through high resolution complementary and correlative microscopy techniques.

1. The changes in form of a human tooth relative to the socket-shape and vice versa can prompt local adaptations through strain concentrations within the PDL, PDL-bone and PDL-cementum interfaces that facilitate the dynamic nature of the bone-PDL-tooth fibrous joint function (8, 31)

The mammalian tooth attachment is a part and parcel of the oral and craniofacial masticatory complex, possesses the unique characteristic of short range motion compared to long range motion observed in diarthrodial joints of the musculoskeletal system. Such short-range motion is provided by a soft, fibrous PDL that attaches cementum to the alveolar bone, and acts as a shock absorber to distribute occlusal loads during mastication (11, 32, 40). Absorption of functional loads decreases hard tooth-tooth impact and enamel wear, alleviates stress/strain concentrations across PDL-cementum, PDL-bone attachment sites and interfaces, and allows for decades of cyclical loading (40, 41). Tooth movement due to chewing within physiological limits drives joint function through maintenance of the functional PDL-space via mechanobiological processes (42). The goal of this section is to elucidate tooth movement within the alveolar socket and to gain insights into the contribution and adaptive capacity of the constitutive properties of soft/hard tissues and interfaces to maintain joint biomechanics. Experimental approaches included a validated holistic method using noninvasive imaging technique through micro-X-ray computed tomography (μ-XCT) coupled to a mechanical loading device (6, 8). *Ex vivo* experimentation enabled visualization and evaluation of the effect of physical shifts within the intact complex of a loaded fibrous joint (6, 8).

1.1. Entheses, Entheses Organs and Interfaces: Materials Science, Biomechanics, and Mechanobiology Perspectives (Figure 1) (8, 32, 43)—

Similar to other diarthrodial joints in the body, the load-bearing bone-PDL-tooth fibrous joint has the ability to react and sustain cyclic load through self-regulated biological processes. Under loaded conditions, mechanical strains can stimulate cells at various sites of the complex (Figs. 1a and 1b), and promote strain-adaptive properties within periodontal tissues and their interfaces (Figs. 1c and 1d). From a materials perspective, the strain-adaptive properties are termed as physicochemical properties.

Functional aspects related to interfaces within the context of biomechanics and mechanobiology were eloquently stated by Darcy Thompson and were adapted to fit within manuscript's content. Thompson wrote "…and the beauty and strength of the mechanical construction (joint) lie not in one part or in another (tissues per se), but in the harmonious concatenation (interfaces) which all the parts, soft and hard, rigid and flexible, tension-bearing and pressure-bearing, make up together (44)." An interface is a "concatenation" of dissimilar materials. Various analytical techniques, similar to materials science lessons from nature, have taught that an interface can be converted to a homophone; interphase in that it is a region that encapsulates changes in physical and chemical characteristics that permit transition of one material to another (soft to hard (vice versa), hard to hard, soft to soft – all of which are dissimilar tissues) in a seamless fashion. This implies that an interphase is a transition zone and challenges identification of the end and beginning of two dissimilar tissues.

In biology, a term analogous to an interface is an enthesis organ. This term was first used in the musculoskeletal system to define interfaces between dissimilar tissues such as bone and tendon (osteotendinous), or bone and ligament (osteoligamentous) (45, 46). There is yet another term known as the "junction". However, as used in the context of an interface, it is limited in meaning as it alludes to a physical demarcation between two different materials/tissues as seen by the naked eye, or limited to a low resolution light microscope. Several such junctions known as the dentin-enamel, cementum-dentin, and cementum-enamel junctions were also observed (47–52). From mechanics and materials science perspectives, the topic of particular interest to researchers is "fracture toughness" of a seemingly discontinuous system, that is, that which can be defined as an abrupt change from one material to another. However, interdisciplinary approaches, including various analytical techniques and histological analyses indicated natural interfaces to consist of gradients owing to gradual changes in structure, elemental and molecular compositions, and elastic modulus within a finite width between the dissimilar tissues including the cementum-dentin, cementum-enamel, and enamel-dentin regions (47–53). In a similar fashion, fibrocartilaginous joints showed that as tendons inserted into bone, they transitioned through zones containing gradients in organic and inorganic concentrations interspersed with hygroscopic regions (Figure 1). Consequently, it is thought that these gradual transitions in elemental and biochemical compositions provide resistance to functional demands including fracture (48). The importance of a gradual transition permitted by multiple zones is highlighted specifically when it is contrasted with a single zone, indicated by a sharp increase or decrease in organic to inorganic ratio (54, 55). These single zone regions are observed as elastic discontinuities within load-bearing systems with impaired function (Figure 2) (43).

a. The structural integration in tandem with the gradual variation in physical and chemical characteristics at the PDL-bone and PDL-cementum interfaces exist within a bone-PDL-cementum complex (Figure 1) (32): Interfaces continue to be characterized as bioengineered templates upon which tissues can be regenerated to replace injured and/or chronically inflamed regions that impair locomotion. Within joints, interfaces are localized regions and act as “functional machines” due to crosstalk between a multitude of cells and cell-extracellular matrix structural components that work in concert to accommodate functional forces and permit joint motion (56). Within musculoskeletal and oral and craniofacial systems, these interfaces are grossly categorized as 1) indirect fibrocartilaginous, in which tendons ligaments insert into bone through and intermediate tissue type such as cartilage, and 2) direct fibrous entheses, in which tendons/ligaments insert into bone with no identifiable cartilaginous tissue (57). Studies guided by principles from biomechanics and mechanobiology illustrated PDL-bone and PDL-cementum interfaces of the bone-tooth fibrous joint to express characteristics analogous to direct fibrous entheses. Regardless, applying principles of mechanics of materials, amplification of strains within the cells at the soft-hard tissue interfaces compared to adjacent bulk tissues per se renders the tethered ends of the softer tissues to be susceptible to increasing shear and flexural moments that are commonly identified in load-bearing joints with short and long-range motions. However, from a mechanics perspective to prevent catastrophic failure of the joint through fatigue, cells interspersed with increased concentrations of matrix molecules including the adhesive proteoglycans (PGs) at these tethered ends (58) can promote cell migration and permit their adhesion and matrix interactions needed for tissue regeneration and remodeling (2).

PGs are hydrophilic macromolecules that are responsible for resisting the compressive forces through their water-retention characteristics, and sustaining functional loads in the periodontium (35). PGs are secreted by various cells in the PDL to sequester biomolecules and directly and indirectly modulate extracellular matrix formation. PGs have varied function in that their concentration and arrangement within the 5–10 μm region adjoining cementum and alveolar bone is distinctly different from the PDL-cementum and PDL-bone attachment sites (Figure 1). These include regulating adhesion of fibroblasts, cementoblasts, and osteoblasts to adjacent tissues to repair both degraded tissues and the attachment mechanism, and acting as potential modulators of biominerals (3, 17, 18). Hence, it is conceivable that the inherent characteristics of the PG-decorated PDL-cementum and PDL-bone interfaces and partial swelling at the adjoining hypomineralized regions consisting of hygroscopic PDL-inserts are vital for tooth attachment and modulating physiological loads (31–33, 35, 40) (Figs. 1c and 1d). These identifiable gradual transitions from softer to harder tissues (and vice versa) are used as a standard “blue print” to engineer functionally graded interfaces (FGI). The guiding principle of an FGI is to alleviate mechanical strains by distributing them over a wider interface thereby decreasing susceptibility to failure through tears and ruptures (32, 59–61) thus increasing resistance to fracture. This signature of nature’s FGI has been consistently demonstrated across diverse species that include the squid beak, bamboo stem, and vascular bundle of the *Washingtonia robusta* palm (62–64) to name a few. Within each of these species, gradients in mechanical stiffness have developed as an adaptive response in an attempt to reinforce underlying organ structure to resist the external stimulus. As such, interfaces can be then defined as a collection of related tissues at and near the attachment site (enthesis), and develop in response to environmental cues, such as shifts in mechanical strains. Consequently, the stimulated cells attempt to reinforce regions as they react to the biomechanical signals (mechanical strains related to tension, compression, and shear) that stem as function for any organism. The reinforcement can be identified as a multi-zone interface exhibiting a gradual transition, or a single-zone interface exhibiting an abrupt transition from soft to hard tissues and vice versa. These single zone regions are identified as elastic discontinuities within load-bearing systems with impaired function (Figure. 2).

b. Deviation from measured normal PDL-space (150–380 μm) to adapted regions of (5–50 μm) at the original ligament-bone and ligament-cementum attachment are to resist functional demands (Figure 2) (33): When external stimuli that modulate functional homeostasis are altered and include abnormal regimes, irreversible modeling occurs and physicochemical properties change until a new functional homeostasis within a joint is reached (31, 33). Under these conditions, the question is; what is the functional relevance of these locally measured shifts in physicochemical gradients specifically at the PDL-bone and PDL-cementum and bone-PDL-cementum complex? That is, how does this shift in stiffness gradient affect overall joint function?

Under normal conditions, the graded stiffness from PDL to bone and PDL to cementum could vary due to natural physiological tooth movement with age known as active eruption, as well as by biomechanical function such as; masticatory forces due to normal and malocclusion. 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. In support of the mechanics of materials related principle, a higher incidence of inflammation and failure in the form of tears at soft-hard tissue interfaces is commonly observed when PDL to bone and PDL to cementum interfaces are subjected to nonphysiologic loading. Due to the presence of cells, the increasingly strained interfaces become local regulators and form self-governing zones, and are ultimately observed as chronically inflamed sites. These injuries commonly occur with repeated insults/nonphysiologic stimuli or high impact sports, exemplified by development of insertional tendinopathies at the elbow, the Achilles tendon, and the knee (65–67). Such injuries are also known as enthesopathies, gradually formed at the soft-hard tissue entheses organs/interfaces with continuous nonphysiologic cyclic loads which over time can impede joint mobility and cause joint impairment (68).

In a human bone-PDL-tooth fibrous joint, these elastic gradients at entheses were found to be caused by a gradual increase in inorganic to organic ratio as the softer PDL transits into stiffer bone and cementum (32, 40, 49, 69, 70) (Figure 1). 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) to higher values observed in alveolar bone (0.2–9.6 GPa) and cementum (1.1–8.3 GPa). As a result, under normal function, such interfaces can protect against excessive shear, torsional, bending, rotational, tensile, and compressive forces accompanied with joint movement (59, 71), ensuring optimum load distribution and transmission of cyclic loads (i.e. chewing forces) that prevent wear and tear due to fatigue. However, the following questions are invoked; what would happen should this gradual change in stiffness undergo abrupt change as observed in Figure 2? What would this shift in strain-adaptive properties (in this case the property in Figure 2 is reduced elastic modulus) mean in the context of joint function? And under what external stimuli including clinical scenarios would such a shift in graded properties arise? These questions will be addressed in the following sections.

The adaptive nature of the PDL and its interfaces is thought to be due to vascular elements (blood vessels – BV, Figure 2) and nerves (33) that are continuous with the extracellular matrix of the PDL that consists of fibrous proteins, including the dominant type I collagen, and trace type III and V collagens, elastin and oxytalan fibers, and globular proteins, including proteoglycans and other noncollagenous proteins (72–74). Progenitor cells and their differentiated lineages of osteoblasts, fibroblasts, and cementoblasts, reside within the matrix, but are localized in strain-specific regions of the complex (2). The surface layer of alveolar bone adjacent to the PDL consists of an underlying osteoid matrix with maturing inorganic minerals in the predominant forms of hydroxyapatite and carboxyapatite (75, 76). Typically, the general structure of an alveolar bone consists of an outer dense shell of compact bone known as the lamina dura to which a heavily vascularized, spongy cancellous bone is attached. The thickness of the compact bone as well as size and number of trabeculae in cancellous bone can increase and/or decrease in density in response to hyperfunction and hypofunction, respectively (77–79), and is a controlled by magnitude and duration of loading. These remodeling aspects of bone occur through an orchestrated effort of osteoblasts of mesenchymal and osteoclasts of hematopoetic origins, creating reversal lines caused by deposition and resorption activities, respectively (80–82). Hence, from a functional perspective, one can ask, do these protrusions in bone (Figure 2g) with a higher elastic modulus cause an elastic discontinuity in bone despite dominance in hygroscopic PDL-inserts? And does this discontinuity within the tissue and in the joint shift joint biomechanics from physiologic to pathologic or maladapted function?

Alveolar bone along with its bundle bone protrusion works in concert with another mineralized tissue, cementum within a bone-PDL-tooth fibrous joint. Opposite to the PDL-bone attachment site of the complex, a mineralizing matrix known as the cementoid also exists at the PDL-cementum interface (2, 5, 32, 83). Cementum is thought to be similar in structure to bone; however, it possesses an acellular zone in the anatomical coronal half to two-thirds of the tooth root (5, 83). Deposition and resorption of mineral also occur in cementum as they do in bone; however, these changes are usually in the form of modeling as opposed to remodeling (5, 84). Most often it is thought that cementum is lamellar, does not adapt, and has minimal adaptive response. While this could be true when compared to vascularized bone, results from our laboratory have indicated that cementum could have a delayed response. If so, could this be the reason why bone grows into the PDL-space (Fig. 2b, 2d and 2f), while cementum could subsequently make way to accommodate bone growth? Both these events need not be seen in the same sectioned two-dimensional (2D) plane, and indeed occur in three-dimensional (3D) volumes of the bone-PDL-cementum complex (37). And could this be the reason why secondary events such as conforming or nonconforming forms between the bony alveolar socket and cementum of the tooth can occur?

1.2. Physiologic and Pathologic Strain Amplifications at the PDL-bone and PDL-cementum Interfaces: Conforming and Nonconforming Alveolar Bone-Tooth Surfaces (Figures 1 and 2) (31, 33)—In musculoskeletal and dental orthopedics, conforming and nonconforming surfaces can define the form-function behavior/adaptation of a joint, and provide insights into the local behavior at a tissue level and resulting cellular responses and subsequent genetic and molecular expression. These hierarchical length-scale events provide a continuum-like effect to address functional homeostasis of a joint. Based on the fundamental seminal concept by Julius Wolff, the long-term effect of functional aberrations at the macroscale could result in pathological deformations to maintain function. Over time pathological deformations of mineralized tissues change the internal architecture, as the joint continues to function. Extending this to bone-PDL-tooth fibrous joint, the relative motions between members through a combination of soft and hard tissues and their interfaces will continue to adapt tissues locally. This adaptation is guided by the principles of joint biomechanics intertwined with mechanobiology of tissues. Adaptation is identified by spatiotemporal changes in physical and chemical properties of soft and mineralized tissues including their interfaces which in turn can result in an overall change in a form-function relationship, commonly termed as functional or biomechanical adaptation.

This section will elucidate the effect of eccentric loads, loads which do not align with the anatomical axis of the joint as opposed to concentric loads. Eccentric loads are those imposed by parafunctional habits with varying magnitudes and frequencies in which the principle vector is converted into a moment, and can exacerbate functional adaptation between two nonconforming surfaces in motion, specifically at the tethered ends of the PDL-bone and PDL-cementum attachment sites. These adaptations over time can be observed as physical features known as constrictions, and when investigated in the context of function can lead to stress concentrations and impending failure of the once biomechanically efficient joint. Other significant load-induced perturbations include traumatic and therapeutic loads from orthodontic braces all of which can change form-function relationship and decreased functional efficacy of the bone-PDL-tooth fibrous joint which will be discussed in section 2.

c. These constriction sites within the PDL-space over time are new load-bearing sites and could eventually impair joint function (Figure 2) (31): Form modulates biomechanical response of individual structural components of the fibrous joint including its interfaces to facilitate optimal tooth movement (31) under physiologic and nonphysiologic conditions, including therapeautic loads. The contextual information of interfacial biomechanics is best highlighted by performing *in situ* experiments through visualization of interfaces at no load and under loaded conditions. Following image registration/analyses, the deformation within the PDL as a result of concentric loading (vertical load aligned with the anatomical axis of the bone-PDL-tooth fibrous joint) was mapped as shown in Figure 1. A normal PDL-space of 150–380 μm as observed is mechanically strained under uniformly loaded conditions. However, *in situ eccentric* (off centered) loading of the joint illustrated that tilting of the root in the bony socket can occur and induce tissue adaptations, not only at the interfaces, but within periodontal tissues per se by converting strain-induced deformation within respective matrices (mechanical energy) into biological processes (chemical energy). This conversion predominantly occurs intracellularly, and is facilitated by matrix-cell, and cell-cell interactions (32, 33, 85–88). Specifically, mechanical strains can be localized in regions where nonconformity between the surfaces of the tooth and the alveolar socket is appreciated (Figure 1a – red patches, 1b–block arrows and white arrows). At these regions it is likely that strains at the tendon/ligament-bone entheses could be up to four times the deformation experienced by the tendon/ligament bulk tissue, with microtears and/or microfractures occurring mainly within the interface (89, 90). As a result, cells within tissues near entheses are subjected to high incidences of strain amplification, subsequently prompting wear and tear at a tissue level and causing degenerative diseases called enthesopathies (57, 89).

Enthesopathies can occur as a result of disease and/or load-induced perturbations of higher frequencies and magnitudes of cyclic mechanical forces. The shift in strain within the PDL-space simply due to load (Figure 1a and 1b) and other aberrant inputs, such as bacterial insult, changes in food hardness, and therapeutic loads, can in turn shift the overall displacement of the tooth relative to the socket in response to load, thereby altering the self-regulating biomechanical cycle of the joint and triggering its adaptive nature in perpetuity (Figures 1–3). In other cases, proinflammatory factor expressions, cellular recruitment, and metaplasia at sites of entheses in response to excessive extraneous loads and/or prolonged inflammatory conditions have been shown to propagate the development of bony spurs that discourage joint mobility (56, 57). Hence, prolonged perturbations in the form of excessive extraneous loads often cause chronic inflammatory diseases that could alter or break down tissues involved in constructing stress concentration-relieving entheses organs. The observed bony protrusions could occur due to nonphysiological functional demands at the tethered ends of the PDL-bone and PDL-cementum attachment sites, and strengthen the region to accommodate functional demands. This compromised PDL-space will continue upon further loading, as the constriction sites will become the new "load-bearing sites" that eventually cause direct local fusion of bone with cementum. Similarly, it is speculated that altering function of the fibrous joint, i.e. tooth movement, generates adaptation of tissue composition to address changes in biomechanics (Figure 2 – differences between normal and a compromised human bone-PDL-tooth complex). Clinically, while a narrowed PDL-space can be considered asymptomatic when superimposed with other aberrant loads and clinical interventions it can cause failure of the fibrous joint.

2. *In vivo* models can be used to manipulate and help understand the adaptive capacities of entheses/interfaces. "Natural intelligence" of interfaces can be determined by extracting mechanoresponsive signals from cells within local regions specifically within the context of a clinical setting such as orthodontic tooth movement (OTM) (Figure 3) (9, 37, 38)

In mammalian species, function-related thresholds are most often modulated by food hardness (7, 34, 91–94). Previous studies have shown that the width of the PDL-space changes in response to magnitude and direction of occlusal loads (7). Hyperfunction during which force exceeds physiologic loading (increased magnitude and decreased frequency) has shown to increase PDL-space through an upregulated osteoclastic response, while conditions of hypofunction (decreased magnitude and increased frequency) illustrated modeling of the PDL width to a narrower space than under control conditions (17, 34, 78, 83, 95). Here lies an intriguing aspect of plausible mechanobiological differences between PDL-bone and PDL-cementum interfaces, in that adaptation to magnitude and frequency of loading at bone interface could be significantly different from that which occurs at the cementum interface, and respective tissues alone. This concept will be revisited under the clinical scenario of orthodontics, an extrapolation of fundamentals from structural engineering/orthopedics that states that the anatomical axis and loading axis should coincide (concentrically loaded) for physiological function of a joint. In its absence, the net shift in mechanical strain can prompt increased pullout forces at the PDL-bone and PDL-cementum attachment sites causing cells to differentiate and undergo different rates of durotaxis and haptotaxis. These semiautonomous biological events lead us to propose that the natural plasticity of these tissues continues to guide the organ within physiological limits, but significant shifts in mechanical strain contributed by a change from concentric or eccentric loads or vice versa may result in closing or widening of the PDL-space. These physical transformations resulting from mineral formation and resorption related events at the ligament interface can guide the complex to a nonphysiologic regime. This concept will be illustrated by presenting a clinical scenario, namely orthodontics.

d. The semiautonomous events at interfaces guide the joint within physiological limits, but significant mineral formation and resorption at the PDL-interfaces may result in closing or widening of the PDL-space and can guide the complex to a nonphysiological regime (Figure 4) (9, 37, 38)—There exist many clinical interventions in skeletal (distraction osteogenesis), and oral and craniofacial orthopedics (cranial grafts, orthodontics) that involve the use of mechanical forces to "mold" and/or regenerate bone and its adjacent tissues. However, very little is known about the influence of mechanical stimulus on the biomineralization of tissue per se within the bone-PDL-tooth complex or functional interfaces between 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, and 3) the absence of PDL severely limits the extent of bone remodeling (96) and tooth movement.

The importance of functional adaptations of the bone, in particular at the 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 response to strain density within the softer matrix (97). The effects of strain density within the PDL and PDL-interfaces on biomineralization within the bone-PDL-tooth joint can be correlated to the cellular and tissue adaptations in a mouse model. The specific sites that are mechanically stimulated include the PDL-bone and PDL-cementum functional attachment sites. Due to the nature of the ligament-bone interfaces in the complex, i.e. disparate tissues interfacing over a distance of 10–50 μm, these sites are mechanically strained (45, 61) and the rate of adaptation at the interfaces is higher compared to other modeling sites. For this reason, 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.

In humans, fundamental components for OTM include force, moment arm, and frequency of loading. The effect of these components when observed from cone beam computed tomography scans before and after OTM of the same patient, registered about the center of rotation of the skull, revealed vectors both translation and rotation of teeth (Figure 3). Fundamentally, from a structural engineering perspective, the principle vector under normal conditions is converted to a moment under eccentrically loaded conditions, and as a result is thought to accentuate strains, specifically at regions where dissimilar materials are attached. This concept is constantly leveraged in OTM or experimental tooth movement (ETM) in rodents using various methods of eccentric load placement including the use of an elastic spacer (Figure 3e) to amplify strains at the functional interfaces and attachment sites of the PDL-bone and PDL-cementum. While applied force does lead to gross tooth movement, and perhaps shifts in rates of biomineralization at the ligament interfaces, it is important to consider that bone modeling and drifts that prompted the overall form of the tooth-socket are an attempt to maintain optimum biomechanics needed for continued chewing. Worthy of note is the growth of bony fingers along the strained fibers of the ligament (Figures 4c–4i); a growth not observed at the ligament-cementum interface within the same 2D spatial field (Figures 4d, 4f–4i). The intriguing aspect of plausible mechanobiological differences and resulting adaptation to shifts in magnitudes and frequencies at the PDL-bone and PDL-cementum interfaces can be answered by investigating the genetic expressions of varied cell populations (note the varied cell morphologies commonly identified at strained ligament-bone and ligament-cementum attachment sites, Figures 4h and 4i) at these site-specific volumes within the bone-PDL-cementum complex. Based on this experimental model, several questions follow. Which volumes within the three dimensional (3D) complex should be examined to investigate the mechanobiological effect on cells at these localized regions that act as “functional machines”? That is, where are the semiautonomous regions within the complex located? Additionally, what is the association of genetics with matrix molecular expressions within the same complex? And more from an experimental approach perspective is a 2D field an accurate representation of mechanobiological effects acting on a 3D complex?

Leveraging recent developments in technologies has enabled imaging intact specimens and thereby the 3D bone-PDL-tooth fibrous joint has been subjected to ETM to subsequently provide changes in physical disturbances of the PDL-space otherwise omitted by 2D micrographs. By comparing experimental tooth movement to controls, data provided insights into tooth displacement and strains under ETM, including observations that ETM can exceed distances far greater than PDL-space (Figures 4a and 4b). Undoubtedly, the significantly strained tethered ends of the ligament and its interfaces termed as “functional machines” will elicit cell responses locally. Outcomes of these mechanobiological cell responses are observed as regenerated matrices with physicochemical characteristics adequate of the felt shifts in magnitudes and frequencies at these site-specific regions within a 3D spatial field of the complex. These biological processes are an attempt to restore physiologic PDL-space via active mineral formation and resorption at the PDL interfaces, and other regions of alveolar bone and cementum of the periodontium. The rapid shifts prompted by experimental tooth movement can favor rapid deposition of mineral by cells interacting with the naïve yet mechanically strained organic fabric at the attachments sites and interfaces, and can question the “quality” of bone.

Bone quality assumes several flavors. According to American Society for Bone Mineral Research (ASBMR) (98), bone quality is identified as the “turnover, damage accumulation (.e.g. microfractures) and mineralization of the organic matrix, the chemical composition and extent of crosslinking of the organic matrix, bone architecture, fatigue from repeated loading of bone, the rate and direction of deformation of the bone during trauma and is determined by a complex interplay between the amount of mineralized tissue present in the bone, and the extent of biomineralization” (98). For these reasons, it is clear that this dynamic tissue cannot be simply described by a sole parameter, but functional insight if adaptations in bone maintain optimum function or shift the structure to a maladapted state to address functional demands above or below physiologic threshold range should be gathered. Regardless, upon prolonged stimulus (above or below physiologic threshold range), rates of adaptation are increased at the richly differentiating semiautonomous zones of the PDL-entheses and therefore are susceptible to controlled modulation or modulation toward pathology under these exacerbated conditions. Given that the load-bearing complex also contains cementum and its interface with the ligament, different reaction rates (shifts in magnitude and frequency), where resorption and bony finger formations can be thought of as need biological processes in an attempt to ‘correct’ the orthodontically widened PDL-space. These biological processes at the PDL-bone interface can occur at a significantly higher rate than those at PDL-cementum that would prompt external root resorption of cementum.

It can be argued that the pathologic extension of new bone (Figures 4f–4i) in response to ETM creates a more fragile periodontal complex. Concepts extrapolated from physical measurements are in concurrence with existing (99)thoughts ascertaining that any ETM that prompts tooth-shift by a distance greater than its PDL-space will elicit resorption. Conceptually, in ETM, any region that narrows by more than PDL-space requires resorption of hard tissue, in order to maintain a viable PDL-space for tooth function. In humans, the average PDL-space ranges from 150μm to 38Oμm (32), and any OTM shift greater than the physiologic PDL-space will create similar hard tissue resorption not limited to bone, as it can also occur in cementum. This concept should be acknowledged in the clinic when prompting substantial OTM in patients with short or developing roots, as they may experience dramatic root resorption and subsequent tooth mobility/loss. From fundamental science and engineering perspectives, given such excessive strains in the PDL, it is plausible that the physicochemical properties could be significantly different, which would question the functional quality of bone and the load-bearing dentoalveolar complex. From a clinical perspective, regenerated weaker bone due to aseptic inflammation caused by orthodontics could leave patients at a higher risk of bone loss or root resorption if they later develop periodontitis, a form of septic inflammation (Figure 5).

II. CONCLUSIONS

a. Elastic discontinuities in tissues and adaptive nature of joints can impair function (Figures 1 and 2)

Several naturally graded interfaces exist in the dynamic joint of the bone-PDL-tooth complex (32, 48–50, 52, 58). A gradual gradient in stiffness between a soft and hard tissue can adapt to an abrupt gradient to accommodate physiologic or non-physiologic demands on joints. It is this philosophy that was observed to highlight adaptation/regeneration in regions farther away from the site of injury/insult (which normally occurs closer or on the crown) within the context of joint function (33, 43).

b. Form and function can explain the “plastic” nature of a fibrous joint in humans (Figures 2 and 3)

The alveolar bone of a human jaw is minimally interrogated from a functional perspective. Studies presented to date on tissue mechanics are seldom evaluated within the context of joint function. We sought a holistic approach with the use of state-of-the-art *in situ* mechanical testing coupled to a micro X-ray computed tomography unit; and identified that bony protrusions within a 150–380 μm functional space of a biomechanically active interface can cause joint malfunction (33, 37).

c. A holistic overview of joint- and tissue-level biomechanics (Figure 3)

With the use of advanced technology, the effect of overall form, and the physicochemical properties of dynamic joint/tissues can be investigated. Through this validated technology, it is possible to establish that joint function is governed by form-mediated strain felt by bone, PDL, and cementum tissues that predominantly makeup the load-bearing complex. The coupled effect of overall form at a joint-level and strain-adaptive properties at a tissue-level illustrates the importance of functional continuation of the bone-PDL-tooth fibrous joint, and that load-mediated joint adaptation is best understood through a multiscale biomechanics approach (6–9).

d. Differentiating zones and regenerative potential at the soft-hard functional attachment sites and interfaces (Figure 4)

In load-bearing joints, strain amplification occurs at the attachment sites where disparate tissues attach (12, 100). Shifts in mechanical strains due to physiological or nonphysiological (including therapeutic loads) (37) function at the attachment sites and interfaces mediate self-governing zones (2, 37). The spatiotemporally observed biochemical signals (matrix and intracellular proteins) at the attachment sites provide insights to postulate that biophysical signals can modulate mineral formation and resorption related events in the periodontal complex (2). The shift in specificity and localization of biomolecules with development, function, and therapeutic loading is critical for developing “functional regeneration” strategies, and should be investigated with an emphasis on joint function.

e. Multiscale biomechanics approach can provide insights into joint biomechanics, malfunction, and functional regeneration of tissues and interfaces (Figure 5)

A multiscale biomechanics approach (6–8) is important for correlating tissue-level strain-adaptive properties with overall effects of joint form on function and pathology. Elucidating the shift in localization of biomolecules with development, function, and therapeutic loading of the joint is critical for developing “functional regeneration” strategies with an emphasis on restoring physiological joint function.

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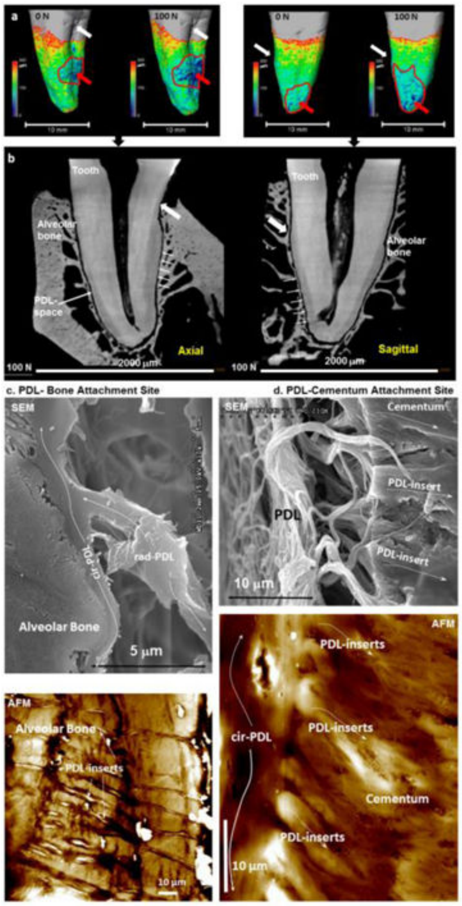


Figure 1. Load-bearing periodontal ligament space (PDL-space), PDL-bone and PDL-cementum attachment sites, and PDL-inserts

(a) Periodontal ligament (PDL)-space represented on the root surface of a human premolar at 0 N (mostly uniform PDL-space) and at 100 N (concentrated as pointed by the red arrow). Axial and sagittal virtual sections in two-dimensional (2D) space (b) are extrapolated from three-dimensional (3D) space (a). White arrows point to equivalent landmarks in both 3D (a) and 2D (b) spaces. Regions with significantly narrowed PDL-space specifically under loaded conditions are encircled in by a red curve (a) and corresponding regions in 2D space are indicated by arrows (b). The attachment of PDL to the alveolar bone (PDL-bone) and cementum (PDL-cementum) as visualized using scanning electron and atomic force microscopy (SEM, AFM) techniques is shown in panel c (PDL-bone) and d (PDL-cementum) respectively. Note a change in radial-PDL (rad-PDL) orientation to circumferential PDL (cir-PDL) as it “skirts” along alveolar bone and cementum before it inserts (PDL-inserts) into respective mineralized tissues within the load-bearing complex. In both cases these inserts are hygroscopic as denoted by their increased height profile when imaged under hydrated conditions (white regions correlate to topographical peaks of 2000–3000 nm compared to darker regions, which correlate to 0 nm).

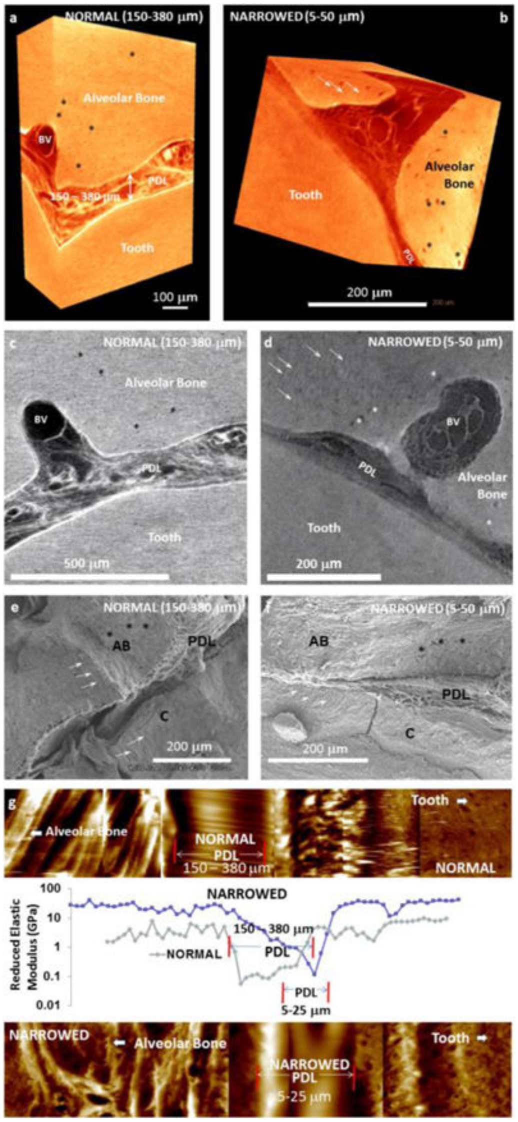


Figure 2. Physical characteristics of a normal and compromised PDL and the bone-PDL-cementum complex using various high resolution imaging and AFM-based nanoindentation techniques

Digitally segmented blocks from human molars illustrate the PDL under seemingly normal (a) and compromised conditions (b). Normal condition is identified as a PDL-space of 150–380 μm (a), and the narrowed PDL-space illustrates 5–50 μm (b). Similar to the 3D digital reconstructions, virtual sections illustrate vascular bundles (blood vessels: BV) within the normal PDL, and similar structures are visible in narrowed PDL (d). Additionally, PDL-inserts can be observed in less X-ray attenuating regions in 3D reconstructed volumes and 2D virtual sections (white arrows in b and d). Similarly osteocytic lacunae are also identified (asterisks in a-d). Scanning electron micrographs of normal (e) and narrowed (f) conditions illustrate PDL between alveolar bone (AB) and cementum (C). Elastic modulus mapping under wet conditions using a nanoindenter illustrate a discontinuity in the narrowed bone-PDL-cementum complex (g).

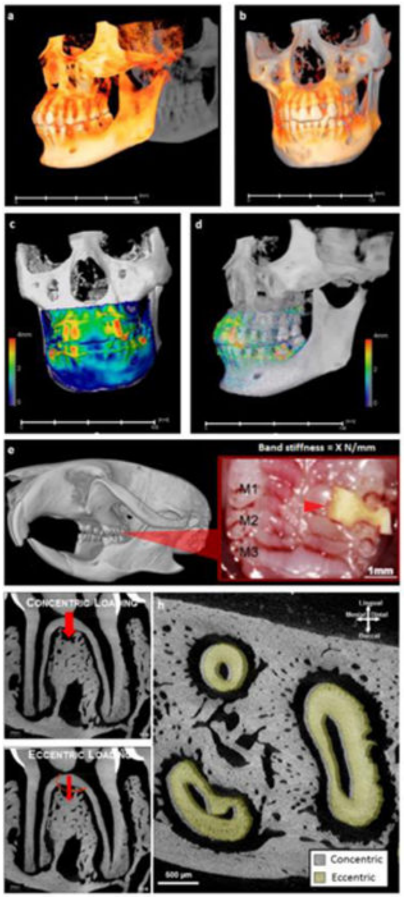


Figure 3. Clinical scenario that exploits manipulating PDL to promote orthodontic tooth movement, and an animal model to link physical perturbations on the crown to biological processes in the bone-PDL-tooth load-bearing fibrous joint

Orthodontic intervention is a clinical scenario that could stimulate plausible discontinuities in physical characteristics of the bone-PDL-tooth complex. Overall tooth displacement by comparing cone-beam computed tomographic reconstructed volumes taken before and after one year of orthodontic intervention is illustrated. Note that observed maximum tooth-displacement is 4 mm and is significantly larger than the 150–380 µm of PDL-space generally identified in humans. Physical changes due to mechanical stimulation resulting from biological processes to prompt tooth translation are programmed in rodents, specifically in mice between the molars 1 and 2 (M1 and M2, red arrow head) with an elastic band of known stiffness (X N/m) (e) to first perform comparative analyses on PDL-space changes (h) under centric (f) and eccentric (g) conditions. Note that in the illustrated scenario the roots of an eccentrically loaded first molar were displaced in the distal direction (h) specifically when compared concentric conditions (g).

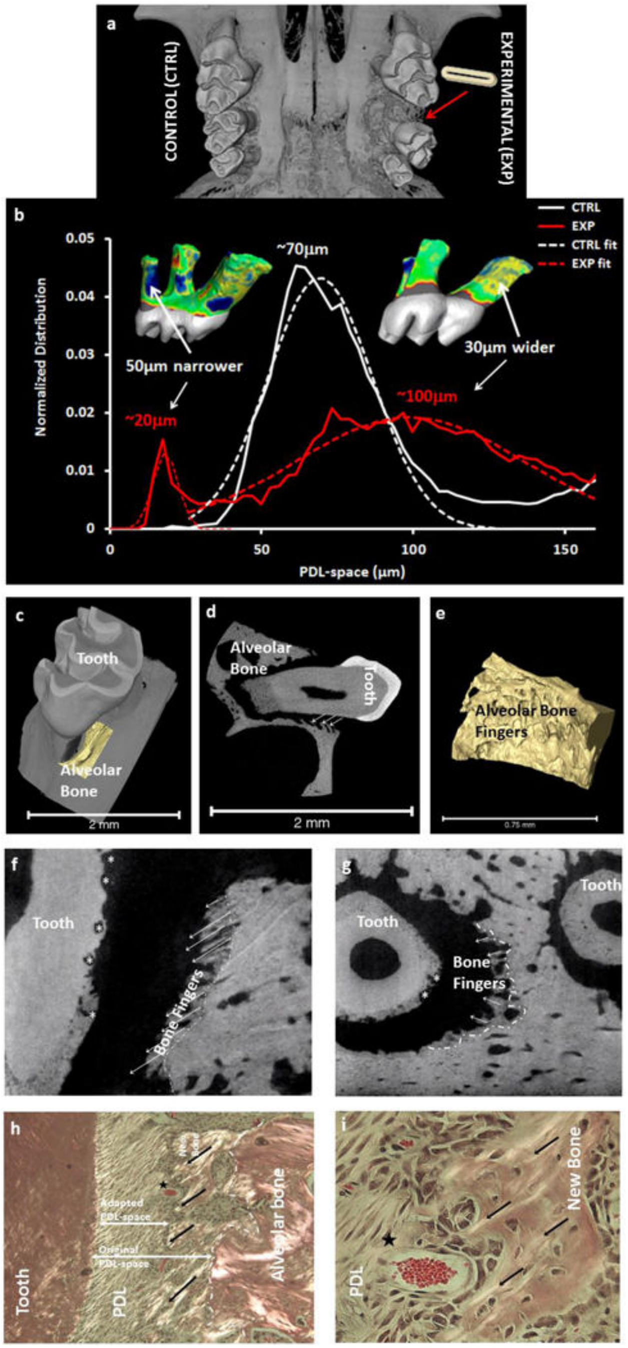
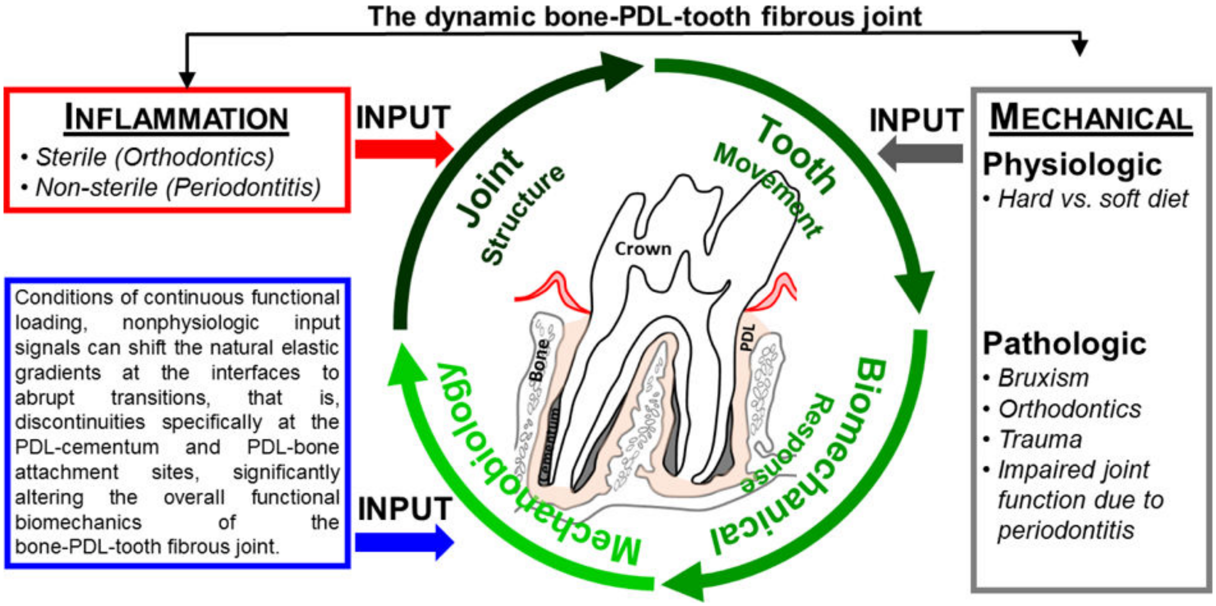


Figure 4. *In vivo* experimental model to program the fundamentals of orthodontic tooth movement to investigate the "intelligence" of the PDL-bone and PDL-cementum interfaces within the bone-PDL-tooth complex

Measured physical changes by comparing the experimental (shows the location and tooth movement due to elastic placement) with control (a) illustrated a homogenous distribution with a peak at 70 µm compared to narrowed (indicated in blue) and widened (indicated in yellow) regions of the PDL-space. The widened PDL-space contributed to bone growth along the strained PDL fibers (b-d, highlighted 3D volume (c, e) and located by arrows in 2D (d) spatial domains). Note the resorption pits in and through cementum invading dentin (asterisks) in longitudinal (f) and transverse (g) directions. Polarized light microscopy on these sections illustrate strained PDL fibers (black arrows) and micro-vessels (star) within regions closer to PDL-bone attachment (g) including hypertrophic cells at the attachment sites (h) compared to polarized cells within PDL *perse* (g, h). Note the differences between original and adapted PDL-spaces (dashed line demarcates original and newly formed bone. Matrix structure is used as a cue to demarcate original PDL-bone attachment site from adapted) as mineral forms on the strained PDL-fibers.



Conditions of continuous functional loading, nonphysiologic input signals can shift the natural elastic gradients at the interfaces to abrupt transitions, that is, discontinuities specifically at the PDL-cementum and PDL-bone attachment sites, significantly altering the overall functional biomechanics of the bone-PDL-tooth fibrous joint.

Figure 5. Adaptation cycle of a bone-PDL-tooth fibrous joint

Encircling green arrows represent the processes involved in joint maintenance and adaptation. The red arrow represents inflammatory perturbations as induced by sterile (mechanical stimulation) and/or non-sterile (bacteria) and other systemic diseases related to metabolic syndromes (diabetes/hypertension) that invariably induce acerbated mechanobiological adaptation by altering the joint structure. The gray arrow represents physiologic and pathologic perturbations that induce strain-mediated adaptation through altered tooth movement in the alveolar socket and plausible impaired joint function. PDL: Periodontal ligament.