Review of the Biological Aspects in the Periodontal Ligament and Alveolar Bone Associated with Compression

REVIEW

Abstract

Background: Tissue remodeling requires protein degradation through a process directly dependent on metalloproteinases - enzymes capable of degrading the majority of proteins in this system. This process is regulated by some enzyme inhibitors, and balance between enzyme and inhibitor activity is fundamental to tissue integrity and health. Bone resorption and formation, and the molecules involved in these processes, raise questions on possible differences in tissue behavior in the system. Histological studies of tissues surrounding teeth that have been subjected to such forces show the presence of tension (associated with bone formation) and compression, which induce bone resorption. The duration of load application is an important parameter in osteoclastogenesis. The present study aimed to analyze the biological aspects associated with in vitro compression of the PL and alveolar bone. To this end, we reviewed articles describing the biological events that occur in the PL and alveolar bone during load application on this system and the possible influence of culture medium on the expression of molecules involved in response to such an event.

Methods: This literature review included articles published between March 2008 and January 2018 describing in vitro laboratory tests and data on load application and its associated biological events in the human PL and alveolar bone, including studies on molecular-expression events and pathways involved in the process.

Conclusions: Intermittent compressive forces stimulate bone resorption in orthodontic treatment, while cyclical forces induce cellular apoptosis at lighter loads than do compressive forces. Both 2D and 3D cell-culture systems seem to reproduce the physiology of evaluated tissues and are reliable analytical models for research purposes.

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Keywords

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Osteoclastogenesis;
Orthodontic Force; Fibroblasts.

Introduction

Periodontal tissues have the capacity to induce and stimulate remodeling and changes in extracellular matrix as a response to application of force to the periodontal ligament (PL) and alveolar bone. Collagen fibers, the main components of extracellular matrix, are responsible for tissue connection [1]. Tissue remodeling requires protein degradation (to allow subsequent protein resorption) through a process directly dependent on metalloproteinases- enzymes capable of degrading the majority of proteins in this system. This process is regulated by some enzyme inhibitors, and balance between enzyme and inhibitor activity is fundamental to tissue integrity and health [2]. The destabilization that occurs when this balance is upset might give rise to pathological situations, including periodontitis, rheumatoid arthritis, cancer, and malformation [3].

Bone resorption and formation, and the molecules involved in these processes, raise questions on possible differences in tissue behavior in the system. For instance, mechanical forces transferred to teeth during mastication are dissipated through the PL [4]. Histological studies of tissues surrounding teeth that have been subjected to such forces show the presence of tension (associated with bone formation) and compression, which induce bone resorption [5].

Cell-biology research often aims to identify differential expression of molecules during different stages of cell differentiation. Additionally, some studies use *in vitro* simulation of *in vivo* tissue physiology, with direct load application on cell [a two-dimensional (2D) system]. For instance, fibroblasts in culture can function as dependent units, with the ability to grow and divide normally as they would *in vivo*, provided the nutrients necessary for growth are available. Fibroblasts require substrate binding for division and growth, and failure in this regard causes proliferation arrest and death [6].

In case of periodontal and peri-implant therapies that, for instance, require an increase in gingival-tissue volume, three-dimensional (3D) culture could

help confirm the findings of studies that have used 2D culture techniques [7]. Tissue engineering comprises three essential elements: culture of appropriate cells; a collagen-, bone-, or synthetic-polymer-based matrix; and addition of soluble mediators containing growth factors and adhesins [8]; the matrices must exhibit structural integrity to form new tissues [9]. These techniques are meant to overcome the limitations of plate- or flask-based cell culture in nutrient cell-culture medium in aspects such as transportation to human receptor sites. Carriers would provide a 3D aspect for cell culture and proliferation and would be ideal for fibroblast support and invasion [10].

While high loads might reduce cell viability and induce necrosis or significant tissue damage, periodontal tissue cells might develop osteoblastic activity when subjected to mechanical stress [11]. Loads applied on this system could stimulate osteoclastogenesis-inducing remodeling, which is responsible for cell differentiation, proliferation, and extracellular-matrix expression [12].

Osteoprotegerin (OPG), receptor activator of nuclear factor κ B (RANK), and receptor activator of nuclear factor κ B ligand (RANKL) are three molecules involved in bone remodeling. They form a part of the RANKL/OPG/RANK system, which plays a key role in bone resorption and can be modified by osteoblasts, cementoblasts, and osteoclasts [13, 14]. The protein RANKL facilitates bone resorption through osteoclasts and activates bone maturation. Osteoblasts may also produce OPG, which acts as a RANKL receptor and subsequently inhibits bone resorption [15].

The duration of load application is an important parameter in osteoclastogenesis [3; 9]. Periodontal-ligament cells have the ability to express cytokines (e. g., during an inflammatory event) that inhibit osteoclast-receptor expression [16]. The present study aimed to analyze the biological aspects associated with *in vitro* compression of the PL and alveolar bone. To this end, we reviewed articles describing

the biological events that occur in the PL and alveolar bone during load application on this system and the possible influence of culture medium on the expression of molecules involved in response to such an event.

Methods

This literature review included articles published between March 2008 and January 2018 describing *in vitro* laboratory tests and data on load application and its associated biological events in the human PL and alveolar bone, including studies on molecular-expression events and pathways involved in the process. In addition, articles on behavior of fibroblasts subjected to cyclic or compressive loads were included.

Articles written in languages other than English and *in vivo* clinical trials were excluded, as were articles that did not evaluate cells experiencing loads or fibroblasts of the PL. The references of articles that met the inclusion criteria were analyzed to identify studies that could be included in the present review.

Results

Upon literature search, a total of 53 articles were identified, of which 5 did not meet the inclusion criteria. The remaining 48 articles were analyzed, of which 36 described experiments involving application of compressive forces. Of these 36 articles, 10 involved experiments conducted using 3D systems (i. e., 3D models for cell culture and load application), and the remaining 26 described the application of the same kind of compressive force on a monolayer system, i. e., directly to cells. Only 10 articles described the application of cyclic force to the PL, all of which used monolayer systems, where in loads were applied directly to cells. The reviewed articles can be found on the **Table 1**.

Table 1. Research articles included in the present review.

Author	Year	Cell culture	Force
Henneman [17]	2008	2D	Compressive
Nakajima [18]	2008	2D	Compressive
Rios [19]	2008	2D	Compressive
Bloemen [20]	2009	2D	Compressive
Kook [21]	2009	2D	Cyclical
Nakashima [22]	2009	2D	Compressive
Sanuki [23]	2010	2D	Compressive
Zhu [24]	2010	3D	Compressive
Nokhbehshaim [25]	2010	2D	Cyclical
Enokya [26]	2010	2D	Compressive
Nokhbehshaim [27]	2011	2D	Compressive
Yamamoto [28]	2011	2D	Compressive
Mitsubishi [29]	2011	2D	Compressive
Diercke [30]	2011	2D	Compressive
Kook [31]	2011	2D	Compressive
Hacopian [3]	2011	2D	Cyclical
Li [32]	2011	3D	Compressive
Nokhbehshaim [33]	2012	2D	Compressive
Drasdo [34]	2012	2D	Compressive
Yu [35]	2012	2D	Cyclical
Deschner [36]	2012	3D	Compressive
Romer [37]	2013	2D	Compressive
Jacobs [38]	2013	2D	Compressive
Barreto [7]	2013	2D	Compressive
Saminathan [39]	2013	3D	Compressive
Li [40]	2014	2D	Compressive
Shen [41]	2014	2D	Cyclical
Konstantonis [42]	2014	2D	Cyclical
Araujo [43]	2015	2D	Compressive
Jianru [44]	2015	2D	Compressive
Sokos [45]	2015	2D	Compressive
Jacobs [46]	2015	2D	Compressive
Yi [47]	2015	3D	Compressive
Aherne [48]	2015	3D	Compressive

Author	Year	Cell culture	Force
Kawase [49]	2015	3D	Compressive
Chen [50]	2016	2D	Cyclical
Ren [51]	2015	2D	Cyclical
Li [52]	2015	2D	Compressive
Wolf [53]	2016	3D	Compressive
Otero [54]	2016	2D	Compressive
Nettelhoff [55]	2016	2D	Compressive
Sheikh [56]	2017	3D	Compressive
Feng [57]	2017	3D	Compressive
Chen [58]	2018	2D	Cyclical
Jacobs [59]	2018	2D	Cyclical

Discussion

In order to elucidate the physiology of the human PL and its behavior under mechanical stresses such as orthodontic movement or mastication, several studies have attempted to create *in vitro* physiological conditions that closely resemble those experienced *in vivo*. A significant number of studies in this regard have been conducted over the last 10 years; consequently, more than one model type has been proposed for evaluation of PL responses under compressive or cyclic load stimuli. In view of the various compression types and cell-culture models of the PL and surrounding bone, we conducted an analytical literature review of different studies and the implications of their results in relation to compressive models applied to human PL fibroblasts.

Three-dimensional systems allow architectural variations, with porosities that allow structural organization and confer biomolecular functionality [60]. This system could optimize cell distribution and enable in depth evaluation of the bone system, PL, and cementum. The use of multiphasic frameworks helps reestablish structural relationships and improve the accuracy of model tissues by

analyzing the most critical points of their structure [61]. Thus, a suitable, rigid, yet porous support would withstand physiological forces and could model tissues of interest. Among currently available options, biodegradable polymers and natural biopolymers are superior for construction of frameworks that mimic the human PL [62, 63]. Among studies included in the present review, the most common frameworks used for *in vitro* PL simulation included porous collagen- [64] and hydrogel-based [65] frameworks.

In addition to structural support, 3D systems provide strong cell-medium adhesion, ensuring a more homogeneous dissipation of force around the entire cellular structure than seen in 2D systems. Thus, 3D systems possibly increase cell viability and system integrity [66]. Jianru et al. [44] showed that PL cells grown over a poly-L-glycolic acid–poly-L-lactic acid matrix exhibit significantly decreased OPG/RANKL ratios after 3–6 h under a compressive force, indicating potential induction of osteoclastogenesis. Upon application of a compressive force, OPG expression decreases after 3 h, begins to increase after 6 h, and reaches significantly high levels after 12 h; this regulation of OPG expression indicates suppression of bone resorption. The authors reported that the increase in OPG levels is positively regulated by KN-93, a specific inhibitor of the Ca²⁺/calmodulin-dependent protein kinase [CAMK] II pathway, and that static compression might activate this pathway. The results of the study by Jianru and coworkers corroborate those of other studies [10, 40, 66] that also used 3D systems, which indicates that this type of framework regulates the expression of certain molecules, while the different cell-cell contact typologies allowed by these systems induce responses that differ from those of monolayer systems.

Kanzaki et al. [67] and Li et al. [32] demonstrated that the increase in RANKL expression in

PL cells subjected to compressive forces promotes osteoclast formation and, consequently, dental movement. Bloemen et al. [20] proposed that direct cell-cell contact would create a favorable environment for RANK-RANKL binding, thus preventing osteoclast-inducing OPG-RANKL interaction. Nishijima et al. [68] reported decreased or unaffected OPG expression in PL cells subjected to compression in a 2D monolayer system. This is in contrast to the findings of Jianru et al. [44], who reported that mechanical stimuli cause a regulated and significant increase in OPG expression in 3D culture. The delay in increase in OPG expression could explain post-pressure bone formation, which prevents alveolar bone loss and allows dental movement.

The main advantages of 2D cell-culture systems over 3D systems include environmental control and provisions for cell counting and evaluating cell growth. In addition, most monolayer cultures depend on cell adhesion. Monolayer systems appear to be reliable evaluation systems when compared amongst themselves; however, the frameworks of 3D systems elicit responses that are different from those seen in monolayer systems. Thus, further studies are needed to develop reliable and reproducible laboratory models, dependent on the cell type under evaluation.

Orthodontic movement may consist of torque and dental extrusion and intrusion movements, which are directly related to the magnitude of force applied, position, and adjacent structures [69]. Di Domenico et al. [70] showed that, while relatively light orthodontic forces are not associated with tissue necrosis, heavy forces generate high tension that can promote tissue necrosis, where dental movement would induce bone resorption. An ideal load would allow alveolar bone resorption followed by tissue accommodation and remodeling, thus ensuring the integrity of the system. Casa et al. [71] reported that alveolar-bone resorption in an area undergoing compression

would lead to osteoblast and osteoclast activity, resulting in solubilization of minerals and matrix degradation.

Lee et al. [72] investigated the effect of shear forces on human osteoarthritis chondrocyte monolayers. Gene-expression patterns after application of shear stress showed high similarity with those occurring during chondrocyte repair. Moreover, microarray findings suggested a close interaction between shear stress and the pathogenesis of osteoarthritis. The authors explained that these results were attributable to the type of force applied in conjunction with the 2D system used.

Wang et al. [73] investigated whether microRNA 208 is a critical regulator of cardiomyocyte hypertrophy under mechanical stretching. They showed that mechanical elongation significantly increases the expression of both microRNA 208 and the cardiomyocyte hypertrophic protein. On the basis of these results, the authors concluded that cyclic stretching increases the expression of these proteins. In a study using a monolayer system, Li et al. [40] reported that, in a hypoxic environment under conditions of periodontitis or physical stress, the PL regulates cyclical-stress responses through cellular proliferation and osteogenic differentiation, suggesting that this is a robust model for the study of mechanisms involved in periodontitis.

Jacobs et al. [46] were the first to show that the viability, apoptosis rate, and OPG/RANKL expression levels of fibroblast monolayers under traction loads in the presence of bisphosphonates are dependent on the magnitude of force. Light forces and bisphosphonates increase bone-apposition factors, while heavier forces in combination with bisphosphonates stimulate osteoclastogenesis. Lee et al. [9] showed that the frequency and duration of mechanical signals are important factors that might influence the biological response to application of force. In addition, many studies [9, 36, 40] have reported that such biological responses are dependent on the type of force applied

and its variables [including magnitude and duration] and not on the type of culture system.

Studies related to continuous mechanical forces evaluate the response of tension that affects cells to changes in the extracellular environment and assess changes in cell physiology by studying the expression of certain proteins. Cytoskeletal components may have distinct mechanical functions in cells [7]; as a response to force, the cytoskeleton may undergo structural alterations to resist further deformation and reorganization to maintain cellular vitality [74]. The matrix, too, receives a load that induces integrin clustering and activation, thus regulating the intracellular-signaling pathway associated with cell proliferation. These are cellular mechanosensors that might trigger signals leading to modulation of gene expression [4].

It is still unclear to what level cells might distend when subjected to different mechanical stimuli [75]. Wescott et al. [11] evaluated the effect of deformation on cultured fibroblasts and subjected them to different types of stretching using a Flexercell strain system. Shear stress can arise during traction and compression, probably because of the fibrous structure of the connective tissue and uneven surface of the alveolar bone [76]. Brown [77] showed that centrifugation can be used to mechanically stimulate and apply fluid shear stress to cell cultures in flasks. The author explained that cell damage is an important issue when studying cellular responses to applied mechanical forces and that cell viability should be measured under all conditions. Appropriate force ranges are selected for a cell viability of 90%, and trypan-10 blue-dye exclusion is the most commonly used test for cell viability.

Howar et al. [78] described an increase in type-I collagen levels in fibroblasts exposed to intermittent biaxial deformity. In contrast, Hacopian et al. [3] showed that the expression of type-I collagen remains unaffected upon application of intermittent mechanical forces. These differences in results are

probably attributable to the different types of force used in the two studies. The study considered intermittent force as more suitable for orthodontic movement than continuous force, since periodontal tissue can repair itself after some period. In the study by Howar et al. [78], cyclical and continuous traction forces caused a statistically significant increase in collagenase production in order of magnitude.

Some studies have raised questions about whether cyclical forces induce higher apoptosis rates than do other methods of application of force. Robbling et al. [79] reported that the mechanosensitivity of cells is restored a few hours after loading and that the expression levels of type-I collagenase and matrix metalloproteinase-1 are positively regulated after fibroblast stimulation with continuous centrifugal force, which helps decrease the risk of high rates of apoptosis. In this system, cells are less sensitive to forces applied at the beginning of the next cycle because the stimulation mechanism decreases after inter-activation rest periods.

Chiba et al. [80] showed that changes in microtubule orientation in human PL cells in response to cyclical elongation could represent a mechanism of self-protection. Cyclical force appears to be a reliable and reproducible stimulus model. Several studies have shown that this type of force more closely resembles that exerted by *in vivo* physiological mechanisms. However, there is a need to evaluate cell vitality, since this mechanism might cause high rates of apoptosis depending on the cell type.

In orthodontic treatment, intermittent compressive force stimulates bone resorption. However, while moderate compressive forces enable active tissue remodeling and dental movement, heavy compressive forces should be avoided in order to prevent tissue damage. Both monolayer and 3D systems seem to reproduce the physiology of the evaluated tissues, but this ability is directly related to the cell type and parameters evaluated in the system.

Conclusions

Intermittent compressive forces stimulate bone resorption in orthodontic treatment, while cyclical forces induce cellular apoptosis at lighter loads than do compressive forces. Both 2D and 3D cell-culture systems seem to reproduce the physiology of evaluated tissues and are reliable analytical models for research purposes.

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