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3 **The Role of Components and Molecules of Periodontal Ligament in**
4 **Orthodontic Tooth Movement**
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8 **ABSTRACT**

9 Orthodontic treatment requires moving and aligning the teeth into a favorable position
10 aesthetically and functionally. To achieve that, the teeth are subjected to forces to push them to
11 their new position. Loading of force on the crown causes tipping of tooth, subsequently the
12 periodontal ligament is compressed adjacent to the alveolar bone on the side toward which the
13 force is directed. On the opposite side of the root, the periodontal ligament is stretched and
14 undergoing tension. Blood vessels are compressed and blood flow is decreased on the
15 compression side, hence less oxygen supply is received on that side. Compression and tension
16 are triggering specific signaling factors and mediators, which create local environment and
17 gradients to regulate remodeling of the periodontal ligament and bone for tooth movement. In
18 this review, we highlight the structures and mediators released in the very narrow zone
19 periodontal ligament during orthodontic tooth loading.

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21 **Keywords:** cytokines, orthodontic, periodontal ligament, tooth movement.

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25 **1.    INTRODUCTION**

26 The periodontal ligament (PDL) is a specialised connective tissue apparatus, located between
27 the cementum of the tooth root and the bone forming the alveolar socket (alveolo-dental
28 ligament) (1) . It acts as a shock absorber which transmits the biting forces to the alveolar bone.
29 Cementum of the tooth root has intrinsic fibres (Ebner fibrils) run horizontally in a circle around
30 the root and extrinsic PDL fibers (Sharpey's fibers) that are inserted into the cementum in one
31 side and to the alveolar bone of the socket in the other side (2). The extracellular matrix of PDL
32 is composed of a network of fibrous structural proteins embedded in amorphous ground
33 substance surrounding cells (3).

34 The ground substance of PDL is 70% water and is thought to have a role in absorbing stress
35 loads applied on the tooth. Tissue fluids tend to increase within the matrix of the PDL ground

substance in case of injury or inflammation. PDL matrix contains several noncollagenous matrix proteins released from the blood vessels passing through the ligament or produced locally by resident cells, these include alkaline phosphatase(4), proteoglycans (5), glycosaminoglycan, glycolipids and glycoproteins such as undulin, tenascin, and fibronectin (6). The cells of periodontal ligament include fibroblasts, osteoblasts, osteoclasts, epithelial rests of Malassez, macrophages, monocytes, mesenchymal or stem-like cells, cementoblasts and odontoclasts, therefore, PDL considered as a cell reservoir for tissue proliferation and repair, homeostasis or regeneration (7-9). Periodontal ligament also contains blood and lymphatic vessels, and nerve fibres. The width PDL ranges from 0.15 in the middle third of the root to 0.38 mm in the coronal and apical third of the root. It has been reported that PDL space decreases progressively with age or in certain bone disease conditions (1).

Regarding the aging of PDL, it has been found that the space between the alveolar bone and the cementum is gradually reduced over lifespan. Though bone formation decreases and bone resorption increases with aging, yet the PDL region tends to be thinner. This is probably attributed to the increased cementum thickness with age on the expense of PDL width. In addition, the reduced or loss of occlusal forces on aging result in osteoporotic alveolar bone and atrophy of PDL fibres, hence a narrower PDL width (10,11).

Orthodontic forces result in structural changes of the dental tissues features. Cementum, PDL, bone, gingival tissues and bioactive substances (chemical mediators, enzymes, growth factors, neuropeptides and ligands) are collectively reacting and responding to the forces, subsequently periodontal tissues are re-organised and movement of teeth can be achieved (12,13).

The levels of these bioactive molecules may be affected (either increased or decreased) by certain medical conditions or medications taken. Unwanted disruption of the levels these molecules will aggravate or suppress the inflammation induced upon loading. If the normal scenario sequence of aseptic inflammation is disrupted, the orthodontic tooth movement may either accelerated, delayed or even halted. Hence, clinically these molecules can be used as targets to control or adjust the teeth movement to achieve the most favorable aesthetic outcome of orthodontic treatment.

In this article, we provide insights into PDL components and bioactive substances released within the PDL region during orthodontic tooth movement.

## 2. MAINTENANCE OF PERIODONTAL SPACE

PDL is occupying a space surrounded by two mineralised tissues (root cementum from one side and alveolar bone from the other side). This narrow space is maintained by molecules secreted

70  by PDL cells that control calcification and aviod the ankylosis of tooth root with the adjacent

71  alveolar bone throughout the life. The activities of these cells in secreting molecules of various

72  types of proteins are increased during orthodontic movement. Certain mineralisation inhibitors

73  such as matrix gamma-carboxyglutamic acid protein, a vitamin-K-dependant molecule;

74  osteogenic transcription factor Msx2 and glycosaminoglycans or tripeptide–cementum

75  attachment protein may have a pivotal role in maintaining the PDL space (14,15). It has been

76  found that balanced and reciprocal activities between bone sialoprotein and osteopontin may

77  play a significant role in preserving and maintaining an unmineralized PDL space. Osteopontin

78  is expressed in the periodontium since tooth root development and has a potential role in

79  periodontal tissue formation and maintaining the PDL - alveolar bone interface (16). Due to its

80  chemotactic activity in attracting osteoclasts precursor, osteopontin has been implicated in

81  hastening tooth movement and root resorption during orthodontic treatment (17-19). It has been

82  reported that ERM has a role in the osteoclastogenesis of the alveolar socket, a process that

83  subsequently leads to maintain the periodontal space during orthodontic treatment (20-22).

84

85  **3.     ADAPTATION OF PDL TO FUNCTION**

86  Teeth are subjected to two types of forces: continuous, light and horizontal forces represented

87  by soft tissues pressure from the tongue, cheeks and lips; and intermittent, heavy and vertical

88  forces produced by chewing (23). The PDL has the capacity to adapt to load changes.

89  Increasing the loads for long term can increase markedly the width and thickness of the PDL

90  fiber bundles. Contrariwise, a decrease in the load subjected on teeth leads to thinning of

91  periodontal ligament and a reduction in thickness and number of the fiber bundles (24) . These

92  load changes of the PDL are taking place concurrently with adaptive changes at the interface

93  with cementum and alveolar bone (25).

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95  **4.     PERIODONTAL LIGAMENT COMPONENTS IMPLICATED IN ORTHODONTIC**

96  **MOVEMENT**

97  Epithelial rests of Malassez (ERM) cells are remnants of the disintegrated Hertwig's epithelial

98  root sheath, are found in the PDL space. TALIC et al. (26) conducted an animal study and found

99  that ERM cells tend to proliferate and increase in size during experimental tooth movement.

100 Orthodontic tooth movement causes an increased level of IL-6 released by human PDL

101 fibroblast (27). IL-6, in turn, promotes the proliferation and migration of ERM (28). ERM

102 proliferation is reflecting the potential role for these epithelial cells in accelerating the collagen

103 turnover in the periodontal ligament during tooth movement (26-28).

It has been shown that ERM and PDL fibroblast have a reciprocal activity based on the singling and stimuli they receive. Periodontal fibroblasts produce collagen and a collagenase inhibitor (29), while ERM produce latent collagenase which subsequently transforms to active collagenase by enzymatic cleavage (30). ERM plays a role in the rejuvenation of collagen in the periodontal ligament. During PDL remodelling, collagenase of ERM degrades the collagen molecule into three-fourth and one-fourth peptide fragments, which then phagocytize by fibroblasts. Consequently, fibroblasts synthesize and secret collagenase inhibitor which inhibits the active collagenase enzyme to form an inactive enzyme inhibitor complex (29,30).

It has been reported that the ERM contributes to the homeostasis of periodontium. ERM is thought to be involved in the induced tooth movement by increasing epidermal growth factor (EGF) production in PDL, preventing ankyloses and helping to repair root resorption areas by inducing cementogenesis (30,31). EGF in return, stimulates ERM proliferation and maintains their growth and integrity (32). EGF receptors are composed of transmembrane proteins that trigger tyrosine kinase intracellularly and initiate cellular events that activate cell division and remodelling/regeneration of periodontal tissue (33). EGF secreted by the ERM is directly involved in osteoclastogenesis of alveolar bone through the suppression of osteoprotegerin, an important decoy receptor for RANKL (34). Continuous release of EGF results in the upregulation of expression of monocyte chemoattractant protein-1 (MCP1), and hence, promoting the resorption of the adjacent alveolar bone, that ultimately leads to tooth movement, prevents ankylosis, as well as, maintaining the PDL space (20,30).

As part of its multifunction, ERM secret inflammatory mediators such as prostaglandins and enamel proteins such as amelogenin and amelin. Amelin, in turn, promotes the fromation of bone matrix proteins, osteopontin, osteoprotegerin, BMP-2 and sialoprotein. These proteins participate in the regeneration and repair of periodontium (35-38). Furthermore, it has been reported that disruption of periodontal integrity by trauma or orthodontic movement results in the expression of amyloid enamel protein APIN in ERM, which indicates that this protein may play a role in the initial phases of periodontal regeneration (39). Mechanical forces induced by orthodontic appliance enhance the expression of bone matrix proteins, such as osteopontin, which stimulates resorption and repair of root and helps to prevent ankylosis (36,38,40).

### 4.1 Inflammatory Mediators, Cytokine And Transcription Factors

The orthodontic treatment induces an acute inflammatory response at its initial phase. In the early phase of tooth movement, leucocytes tends to migrate out of blood capillaries of periodontal ligament and producing cytokines which subsequently promote the excretion of

138    prostaglandins and other mediators (41,42). Prostaglandins, which are also released by ERM,

139    specifically prostaglandin E2, induce the recruitment and activation of osteoclasts and stimulate

140    bone resorption/remodeling (20,30). Application of orthodontic force stresses the extracellular

141    matrix and deforms the nearby osteocytes of alveolar bone. Subsequently, this deformation

142    opens the hemi-channels of the stressed osteocytes to release prostaglandins and recruit

143    osteoclasts, which enhance orthodontic tooth movement (43,44). Therefore, amount of

144    prostaglandins produced plays a key role in orthodontic movement and also affects the rate of

145    tooth movement (45,46).

146    Orthodontic forces enhance the production of various cytokines within the PDL space. Previous

147    studies highlighted the role of IL-1β, IL–6, and TNF-α in bone remodelling (47,48,49). The

148    concentration of these cytokines is increased in the gingival crevicular fluid at the beginning

149    stage of orthodontic treatment (12th and 24th hour) (50). The cytokines are released by the

150    infiltrating polymorphonuclear leukocytes and PDL cells in response to the applied forces (51).

151    The maximum level of the released molecules is attained three days after the application of

152    orthodontic loading (52). Cytokines induce local stimulation of osteoclasts which perform bone

153    resorption (53,54). The process involves nuclear transcription factor Kappa B (NF-κB) (receptor

154    activator of NF–κB ligand, RANKL) which activates receptors of TNF–α (TNF–R1) at osteoblasts

155    (55,56). RANKL binds on a receptor at osteoclast named RANK. Subsequently RANKL

156    activates osteoclasts to perform osteoclastogenesis (57). Resorption is suppressed through

157    prevention of RANKL binding for RANK by the decoy receptor osteoprotegerin, followed by the

158    inhibition of osteoclasts differentiation (58,59).

159    This phase of alveolar bone resorption is shortly followed by decreasing the levels of secreted

160    pro-inflammatory cytokines and reduction of blood vessels permeability (60).  As cytokines

161    release is diminished in the tissues, the levels of these cytokines in gingival crevicular fluid and

162    the number of inflammatory cells is reduced after 7–10 days since the commencement of the

163    orthodontic forces application (52,61). The later events overlap with the beginning stage of

164    regeneration/repairing of tooth supporting tissues, which lasts for around 9 days (62). During the

165    restoration of periodontal tissue, stimulation of osteoblasts takes place through the

166    overexpression of various growth factors and interleukins which enhance the osteoid formation

167    and suppress bone resorption (63,64).

168    During the early stage of orthodontic loading and after the initiation of early stage periodontal

169    remodelling, there is a second wave of other cytokines such as IL-8 which was shown to have

170    an increased level in the gingival crevicular fluid (6, 45). IL-1β, IL-6, and TNF-α stimulate the

171    production of the other cytokines in monocytes, macrophages, epithelial cells, and fibroblasts of

172 periodontium, this in turn, provoke the release of another wave of IL-1β, IL-6, IL-2 and TNF-α
173 (65,66).
174 High mobility group box 1 (HMGB1), a late inflammatory cytokine, is secreted by the periodontal
175 ligament cells in response to mechanical stimuli (67).  It is also produced by stimulated dendritic
176 cells, necrotic cells, macrophages and monocytes as a cytokine mediator of Inflammation
177 (68,69). On compression, HMGB1 provokes local inflammatory responses by stimulating the
178 secretion of chemokines and cytokines from stressed cells (70,71). The released HMGB1,
179 would, in turn, stimulates human monocytes to produce and release TNF, IL-1α, IL-1β, IL-
180 1RA, IL-6, IL-8, IL-10, macrophage inflammatory protein (MIP)−1alpha, and MIP-1beta in
181 cultured periodontal ligament cells (70,71). Furthermore, HMGB1 induces mnocytes
182 chemotaxis, macrophage migration and osteoclastogenesis (69,72). HMGB1 is produced in the
183 tension zone of PDL during orthodontic tooth movement, where it plays a role in tissues
184 remodelling through stimulation of cells proliferation and formation of mineralized nodule in PDL
185 cells (73,74).
186 Previous study has reported that periostin, a 90 kDa extracellular matrix protein, has an
187 inhibitory effect on HMGB1 (75). Periostin has a potential role in preserving the integrity of PDL
188 collagen fibrils during orthodontic tooth movement, and its deficiency causes impairment of
189 collagen fibers degradation at the compression side of periodontal ligament, which obstructs
190 orthodontic tooth movement (67).
191 Periostin is primarily expressed in connective tissues subjected to mechanical stress and
192 stretch, such as skin, heart valves, PDL, bones and tendons (76-78). Xᴜ et al. (79) found that
193 tensile stress load upregulates the levels of periostin in animals and human periodontal ligament
194 fibroblasts during orthodontic tooth movement. Previous studies have  also found that the
195 periostin expression is regulated by transforming grown factor β, and that periostin promotes
196 fibrillogenesis in PDL; and enhances migration of fibroblasts and osteoblasts, hence, it is
197 essential for the PDL and bone remodelling during orthodontic loading (80,81).
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199 **4.2    Heat Shock Protein**
200 Orthodontic force disturbs the blood supply of periodontal ligament and leads to hypoxia
201 and ischemia in the tension zone during the early stages of tooth movement [(82).
202 Consequently, these disturbances lead to the activation of cellular self-defense activities to
203 reduce the stress and preserve the structure of periodontal ligament. It has been reported that
204 heat shock protein (HSP) is released to serve this mission. HSP is upregulated in periodontal
205 ligament during the early stages of orthodontic tooth movement (83-85).

Previous studies revealed that HSP70 has a regulatory role in the reduction of cytokines and RANKL expression and controlling the inflammatory periodontal ligament cell response to compression forces (84,86). Inhibition of HSP70 resulted in significant reduction in cell division and proliferation and wound healing; while necrosis and apoptosis, osteoclastic differentiation and monocyte adhesion were highly increased in order to limit the tissue damage during orthodontic tooth movement (87).


### 4.3    Periodontal Ligament Blood Vessels

During orthodontic tooth movement, periodontal ligament blood vessels are significantly involved in the renovation of tooth surrounding tissues (3,88,89). Several signals are generated after the compression of extracellular matrix which surrounds the cells of endothelia of blood vessels. These signals activate the restructuring of existing vessels and also formation of new blood vessels in the periodontal ligament (90).

Mechanical forces cause distortion of the nerve terminals which in response they release vasoactive neurotransmitters (91). In the periodontal ligament, most terminals are near blood-vessel walls. Subsequently, the released neurotransmitters activate the capillary endothelial cells receptors to bind circulating leukocytes, enhancing their migration out of the capillaries (92). The migrating leukocytes secrete many signal molecules, including cytokines and growth factors, which play important role in the aseptic inflammatory reaction that stimulates periodontal ligament and alveolar bone remodeling and facilitates the movement of teeth (43).


### 4.4    Periodontal Ligament Cells Role In Osteoclastogenesis

It has been shown that periodontal ligament fibroblasts are adapted to the bacterial invasion and mechanical loading by secreting high amounts osteoclastogenesis-inducing factors. Hence, they possibly contribute to the escalated osteoclast recruitment observed during periodontitis and to orthodontic tooth movement (54). It is believed that periodontal ligament cells regulate osteoclast differentiation through RANKL stimulation and osteoprotegerin inhibition, and also support osteoclastogenesis through cell-to-cell contact (93). Cell-cell adhesion between periodontal ligament cells and osteoclast precursors significantly enhances the upregulation of genes for osteoclast differentiation and the eventual formation of osteoclasts (94).

**4.5    Periodontal Ligament Cells Role In Osteoblastogenesis (Osteogenesis)**

Periodontal ligament fibroblasts subjected to tensile strain were induced to express ephrin-B2 protein which in turn stimulate the osteoblasts of the alveolar bone and increase their osteoblastogenic gene expression at the tension sites during orthodontic tooth movement (95). At the compression site of root, compressive forces induce periodontal ligament fibroblasts to produce ephrin-A2, while the expression of ephrin-B2 fet is down-regulated. Ephrin-A2 suppresses osteoblastogenic gene expression (RUNX2, ALPL) of osteoblasts and decreases the signs of osteoblastic differentiation at the compression sites (95,96).

**4.6    Growth Factors**

Orthodontic loading alters the flow of blood in the periodontal ligament and leads to the production of the angiogenic regulator,  vascular endothelial growth factor (VEGF), and tissue growth factor β (TGF-β) by the periodontal ligament fibroblasts and circulating leukocytes (97,98). It has been shown that the level of anti-inflammatory cytokine TGF-β is higher at the tension side compared to the compression side of the root, which is probably attributed to its role in the process of osteogenesis and tissue formation at the tension side (3,41).

**4.7    Enzymes**

The inducible nitric oxide synthase (iNOS), endothelial nitric oxide synthase (eNOS) and neuronal nitric oxide synthase (nNOS) are three important enzymes released in the periodontal space during the early phase of orthodontic loading (99,100). These enzymes are necessary for the production of nitric oxide (NO) which is an important regulator of bone remodelling (101,102). Previous studies revealed that stimulation of NOS increases tooth movement, while inhibition of NOS reduces that movement (103,104). The gene expression of synthase enzymes is controlled by pro-inflammatory cytokines (IL-1β, TNF-α) and anti-inflammatory cytokines (IL-4, IL-10, TGF-β), which are secreted during tissues remodelling (105). Orthodontic loading on animals have shown that iONS is associated with bone resorption at the compression zone while eNOS is associated with  the bone formation at the tension zone (100).

**4.8    Regulating Occlusal Forces**

The periodontal ligament has a pivotal role in controlling occlusal force associated with muscle spindles in jaw-closing muscles (106,107). There are multiple receptors mediating the somatosensation in the periodontal ligament. These receptors include Ruffini endings and Merkel cells for mechanosensation processing.  Nociception and itching are processed by the

274 free nerve endings of Aδ- and C-fibers (108-110). Merkel cells of the epithelial rest of Malassez
275 release neuropeptides which control the mechanosensation function (108,111). Application of
276 orthodontic forces leads to the constriction of the blood capillaries of the periodontal ligament in
277 the pressure area that result in focal necrosis, with histological features of hyalinization (112).
278 During this process, collagen fibers formation is regulated by ERM in order to promote
279 adaptation to the orthodontic forces, homeostasis and maintenance of the periodontal tissues
280 (12,30,38) .

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283 **5.    CONCLUSION**

284 The periodontal ligament plays a significant role in the orthodontic tooth movement for being the
285 field and source where many bioactive molecules are released in response to the orthodontic
286 forces. Periodontal ligament is the first periodontal structure receives and reacts to the impact of
287 loading. It is also important for being a pivotal part of theater for many molecular events which
288 take place in order to achieve the orthodontic treatment. Clinically, several molecules produced
289 by the periodontal cells upon orthodontic loading can be used as targets to accelerate or
290 improve the movement of teeth.

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293 **CONFLICT OF INTEREST**

294 The authors have stated clearly that there are no conflicts of interest.
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