Inflammation in periodontal tissues in response to mechanical forces

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Summary

Orthodontic forces are known to produce mechanical damage and inflammatory reactions in the periodontium and dental pulp, as well as inflammatory mediators, e.g. prostaglandins, interleukin (IL)-1, IL-6, tumor necrosis factor α, and receptor activator of nuclear factor κB ligand, in the periodontal ligament (PDL) and dental pulp. We have studied the effects of aging on the production of inflammatory mediators in the PDL using in vitro and in vivo methods and found that aging of PDL tissues may be an important factor in the severity of periodontal disease through a higher production of inflammatory mediators in response to mechanical forces. Further, the levels of inflammatory mediators in gingival crevicular fluid, an osmotically mediated inflammatory exudate found in the gingival sulcus, have been shown to be significantly elevated during orthodontic treatment. In order to reduce inflammation, low-level laser therapy has been recently studied in vivo and in vitro by many investigators as a substitute for anti-inflammatory drugs. Clinical and experimental studies have shown that low-level laser irradiation reduces orthodontic post-adjustment inflammation. We believe that orthodontic forces (mechanical forces) may play an important role in periodontal inflammation and that low-level laser therapy may be useful for its inhibition.

Key words: inflammation • mechanical forces • cytokines • orthodontics • periodontal disease

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INTRODUCTION

Prostaglandins (PGs), interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF-α) are key mediators involved in a variety of immune and acute-phase inflammatory response activities. The RANK/RANKL (receptor activator of nuclear factor κB and RANK ligand) pathway is also essential for osteoclast differentiation in inflammatory arthritis.

Orthodontic tooth movement is induced by mechanical stimuli and facilitated by remodeling of the periodontal ligament (PDL) and alveolar bone. A precondition for these remodeling activities, and ultimately tooth displacement, is the occurrence of an inflammatory process. Vascular and cellular changes were the first events to be recognized and described, and a number of inflammatory mediators, growth factors, and neuropeptides have been demonstrated in periodontal supporting tissues. Their increased levels during orthodontic tooth movement have led to the assumption that interactions between cells producing these substances, such as nerve, immune, and endocrine system cells, regulate the biological responses that occur following the application of orthodontic forces.

Mechanical stress evokes biomechanical and structural responses in a variety of cell types in vivo and in vitro. The overall objective of many investigations has been to further the understanding of the mechanisms involved with converting physical stress to the cellular responses that result in tooth movement. In sites where inflammation and tissue destruction have occurred, cells may communicate with one another through the interaction of cytokines and other related molecules. Thus it is important to elucidate completely the complex cytokine cascade flow associated with inflammation-mediated tissue destruction at the molecular level.

Pain is one of the cardinal signs of inflammation, as well as a nearly inevitable and, for the patient, most unpleasant reaction to orthodontic therapy. The incidence of pain may be associated with the interactions of inflammatory mediators. Therefore, control of inflammatory mediators may be important to relieve the pain that accompanies tooth movement. Recently, low-level laser therapy has been shown to inhibit inflammation and, RANKL) in periodontal tissues associated with orthodontic tooth movement. Further, we report findings showing the inhibitory effect of low-level laser irradiation on periodontal tissue inflammation.

INFLAMMATORY MEDIATORS IN PERIODONTUM

Prostaglandins

PGs, a product of arachidonic acid metabolism, are local hormone-like chemical agents produced by mammalian cells, including osteoblasts, that are synthesized within seconds following cell injury. One of the derivatives of the arachidonic acid cascade, PGE₂, acts as a vasodilator by causing increases in vascular permeability and chemotactic properties and it also stimulates the formation of osteoclasts and an increase in bone resorption.

The cyclooxygenase (COX) family of enzymes consists of two proteins that convert arachidonic acid, a 20-carbon polyunsaturated fatty comprising a portion of the plasma membrane phospholipids of most cells, to PGs. The constitutive isoform (COX-1) is found in nearly all tissues and is tissue protective. In contrast, COX-2, the inducible isoform of COX, appears to be limited in basal conditions within most tissues, and de novo synthesis is activated by cytokines, bacterial lipopolysaccharides, or growth factors to produce PGs in large amounts in inflammatory processes. There are several lines of evidence showing that COX is also closely associated with periodontitis, and that PGs are mediators of gingival inflammation and alveolar bone resorption. In addition, numerous studies have confirmed that among lipid mediators, PGE₂ levels in periodontal tissues and crevicular fluid in particular are highly correlated with periodontal tissue destruction. Further, recent studies have suggested that COX-2 is induced by periodontitis and plays an important role in gingival inflammation and alveolar bone destruction.

Cytokines

Some studies have suggested roles for the immune system in the regulation of bone remodeling through cytokine production by inflammatory cells that have migrated from dilated PDL capillaries after the application of orthodontic forces. Cytokines are proteins that act as signals between the cells of the immune system, which are produced during the activation of immune cells and usually act locally, though some act systemically with overlapping functions. Previous studies have implicated the involvement of certain cytokines in bone remodeling in vivo and in vitro, including IL-1, IL-6, and TNF-α, which are key...
mediators involved in a variety of immune and acute-phase inflammatory response activities." 

IL-1 exists in two forms, α and β23, of which IL-1β is the form mainly involved in bone metabolism, stimulation of bone resorption32, 43, and inhibition of bone formation115. IL-1β also plays a central role in the inflammatory process. The staining of feline PDL cells for IL-1β showed the presence of bound signal complexes in the plasma membrane, which was expected, as it is known that receptors for IL-1β are present on fibroblasts25. Lo et al.70 suggested that large amounts of IL-1β are present in inflamed gingival tissues and found that both macrophages and neutrophils are predominant in IL-1β production in inflamed gingival tissues. Kanda-Nakamura et al.54 also suggested that the response of gingival fibroblasts to IL-1 may represent a mechanism for amplification of gingival inflammation. Further, IL-1β may act synergistically with TNF-α24 as a powerful inducer of IL-668, 122.

IL-6, a multifunctional cytokine previously referred to as B cell stimulatory factor 2, hepatocyte stimulating factor, or interferon-β2, is produced by both lymphoid and non-lymphoid cells45 and can apparently induce osteoclastic bone resorption through an effect on osteoclastogenesis50, 59, 73. IL-6 was found to be produced by the human foreskin fibroblast line FS-4 and its synthesis was stimulated by treatment with IL-15, 76. The identification of IL-6 in human gingival tissues and cells implicates this lymphokine as a participant in the molecular events associated with inflammatory periodontal diseases5. Irwin and Myrillas49 reviewed the biological functions of IL-6 and found them to be specifically related to tissue destruction in the periodontal site. In addition, Yakovlev et al.129 found that levels of IL-1β and IL-6 were significantly higher in inflamed as compared with non-inflamed gingival tissues in young adults, while Shimizu et al.104 demonstrated that IL-6 was produced by IL-1β from PDL cells.

TNF-α is a pro-inflammatory cytokine that is often over-expressed in a number of disease states such as sepsis syndrome, rheumatoid arthritis, inflammatory bowel disease113, and periodontitis97, 98, 114. "Takeichi et al.119 found that human polymorphonuclear leukocytes derived from alveolar bone can spontaneously produce IL-1α, IL-1β, and TNF-α in sites of inflammation, and likely initiate inflammation and regulate augmentation of bone resorption in vivo, while Gaspert et al.31 concluded that a subcutaneous administration of TNF-α accelerated the progression of experimental periodontitis in rats. The potential role of TNF-α in osteoclastogenesis is of significance in both tooth eruption and periodontitis. The PDL becomes degraded in periodontitis along with excess resorption of the alveolar bone, and TNF-α has been implicated in this inflammatory condition66.

The possibility that TNF-α is involved in normal physiological processes is supported by its function in osteoclastogenesis. RANKL (RANK ligand, osteoclast differentiation factor, osteoprotegerin ligand) and its receptor RANK, which are present on osteoblasts and precursor osteoclasts, respectively, have been identified as the key factors that stimulate osteoclast formation53, 135. It is now clear that RANKL, together with macrophage-colony stimulating factor, is required for osteoclast formation from precursor monocyte/macrophages43. The natural inhibitor of RANK-RANKL interactions is the soluble TNF receptor-like molecule osteoprotegerin (OPG)137, which binds to RANKL and prevents its ligation, thereby preventing osteoclast differentiation and activation. The importance of these three molecules in regulating bone metabolism has been demonstrated by transgenic and gene knock-out studies in mice58, 90. It is considered that the relative levels of RANKL and OPG in vivo are likely to be important in determining where and when osteoclasts form. Considering the importance of RANK, RANKL, and OPG in physiologic osteoclast formation, it is reasonable to propose that they may also be key regulators of pathological bone resorption. Thus, inflammatory cytokines and the RANK/RANKL/OPG system may act together to trigger bone resorption via regulation of the RANKL/OPG ratio. Previous studies have shown that IL-1, IL-11, IL-17, TNF, PTHrp, and PGE2 increased RANKL mRNA expression by T cells, while both PTHrp and PGE2 decreased OPG expression96, 118.

Recent studies have shown that several cell types may be important in the ectopic production of RANKL in tissues adjacent to bone17, 18, 19, 42. RANKL protein was found to be predominant in inflammatory cells in inflamed tissues adjacent to areas of pathological bone loss in periodontal disease17. Further, a study by Liu et al.69 suggested that up-regulation of RANKL mRNA in inflammatory cells may be associated with the activation of osteoclastic bone destruction in periodontitis. Furthermore, Ogasawara et al.90 reported RANKL mRNA expression in macrophages and multinucleated cells in periodontal diseased tissues in an animal model of periodontitis. Together, the results of these studies suggest that RANKL is also involved in the progress of periodontal inflammation.

**PDL RESPONSE TO MECHANICAL FORCES**

The PDL lies between hard tissues such as the cementum and alveolar bone, where it functions as
a cushion to withstand mechanical forces applied to teeth; thus it receives and responds to external forces. It is likely that PDL cells stimulated by forces of mastication, occlusal contact, and orthodontic treatment produce local factors that participate not only in the maintenance and remodeling of the ligament itself, but also in the metabolism of adjacent alveolar bone.

Several experimental models for mechanical stress have been developed for cell-culture systems, such as application of compressive high pressure, placement of a convex template on the bottom of a Petriperm dish (Heraeus Inc., South Plainfield, NJ, USA), and cyclic tension force controlled by a computer (Flexercell Strain Unit: Flex Corp., PA) (Fig. 1). In addition, in vitro studies have shown that the expression and production of some inflammatory mediators (PGE$_2$, IL-1$\beta$) are promoted by mechanical stimulation of the PDL. COX-2 is induced in PDL cells by cyclic mechanical stimulation and is responsible for the augmentation of PGE$_2$ production in vitro. In addition, Kanazaki et al. demonstrated that compressive force up-regulated RANKL expression and induction of COX-2 in human PDL cells in vitro. These results suggest that PDL cells under mechanical stress may induce osteoclastogenesis through up-regulation of RANKL expression via PGE$_2$ synthesis during orthodontic tooth movement.

In other in vivo studies, experimental tooth movement has been shown to lead significantly to an increased recruitment of cells that belong to the mononuclear phagocytic system (Fig. 2). Saito et al. indicated that there is a local increase in PGs in the PDL and alveolar bone during orthodontic treatment, and other studies have shown an arrest in tooth movement in experimental animals when nonsteroidal anti-inflammatory drugs were administered. Further, when PGE$_1$ was administered locally or systemically to rats, accelerated bone resorption and tooth movement were observed after the application of orthodontic forces. Therefore, PGs play an important role in orthodontic tooth movement.

Macrophages have the ability to produce cytokines, such as IL-1$\beta$ and IL-6, the levels of which are known to increase during orthodontic tooth movement. The number and distribution patterns of RANKL-expressing osteoclasts change when excessive orthodontic force is applied to periodontal tissue, and IL-1$\beta$ and TNF-$\alpha$ were shown to be expressed in osteoclasts in pathological status rat periodontal tissues. Shiotani et al. also showed the presence of RANKL in periodontal tissues during experimental tooth movement of rat molars. Therefore it is suggested that RANKL is regulated by inflammatory cytokines in the PDL in response to mechanical stress.

**RESPONSE OF DENTAL PULP TO MECHANICAL FORCES**

A number of different neuropeptides, including calcitonin gene-related peptide (CGRP) and substance P (SP), are known to be present in the nerve fibers that supply tooth pulp and the periodontium in rats, cats, monkeys, and humans. Further, the morphology and distribution of CGRP and SP...
through immunoreactive nerves have been shown to change their patterns as a result of local pulp trauma, which may indicate that CGRP- and SP-containing fibers take part in the inflammatory process in connection with tissue injury and repair. Orthodontic forces are known to produce mechanical damage and inflammatory reactions in the periodontium, as well as cell damage, inflammatory changes, and circulatory disturbances in dental pulp. In addition, it was recently observed that the expression of CGRP and SP increased in dental pulp in response to buccally directed orthodontic tooth movement of the upper first molar in rats, and that these neuropeptides might be involved in inflammation of the dental pulp at the time of orthodontic tooth movement, while CGRP and SP in dental pulp have been implicated in the mediation of pulpal inflammation.

The peripheral sensory nervous system contributes to the development of acute and chronic inflammatory processes through local release of neuropeptides. SP, a sensory neuropeptide released from the peripheral endings of sensory nerves during inflammation, can modify the secretion of pro-inflammatory cytokines from immunocompetent cells, and has also been reported to induce the secretion of IL-1β, IL-6, and TNF-α from monocytes. CGRP, another major sensory neuropeptide, has also been found to evoke the release of IL-6 and IL-8 from synovial fibroblasts in patients with rheumatoid arthritis. In another study, SP and CGRP both stimulated the release of IL-6 and TNF-α from a human bronchial epithelial cell line. Further, we recently reported that CGRP and SP significantly stimulated the production of PGE2, IL-1β, IL-6, TNF-α, and RANKL in HDP cells. Based on these results, we consider that CGRP and SP may be involved in pulpal inflammation that occurs during orthodontic tooth movement.

### Aging and Inflammation

In general, aging is defined as a decline in the ability to adapt to environmental stress. This decline has often been attributed to a decrease of each cellular function or to an excessive response to stimuli thought to be involved in diseases of the aged. For example, it is well known that the severity of periodontal disease is affected by host age. It is important to define how the aging of periodontal tissue at the cellular level affects the severity of periodontal diseases associated with the aging process, therefore, in vitro and in vivo studies of aging have been conducted.

Fibroblasts have been frequently used as a model for studying the process of cellular senescence. When fibroblasts are allowed to passage in cultures over many generations, they eventually reach a stage where they remain viable but permanently unable to replicate and are generally considered to be senescent. Norwood and Pendergrass expressed the view that though the causes of the loss of proliferative activity of cultured human diploid fibroblasts are unknown, the system is useful as a model for the study of cellular aging in vitro. As a result, methods utilizing increasing population doubling have been used for cellular aging studies. An in vitro model of aging in human PDL cells can be prepared by sequential sub-cultivation (5 to 6 passages for young, 18 to 20 passages for old). Utilizing such models, cyclic tension force-stimulated PGE2 and IL-1β were found to be increased in old human PDL cells compared with younger cells.

Although an in vitro aging experimental system that utilizes an increasing population doubling method is a useful model for cellular aging studies, some points require attention. The changes seen with increasing time in culture might represent either a phenotypic instability of the cells in the culture or the selection of a specific subpopulation of cells rather than true age-related change. However, it remains a useful model that has provided abundant information regarding cell behavior under stressful conditions. Overall, the obtained evidence indicates that in vivo cells accumulate damage over their life-span that results in a gradual loss of differentiated function. Hayek et al. studied in vivo aging using an animal model and suggested that it was useful. In our in vivo aging method, aged PDL cells are obtained from the incisors of 6-week-old (young) and 60-week-old (old) rats and cyclic tension forces are applied. Results have shown that stimulation of PGE2 and IL-1β by tension forces was enhanced by aging in vivo.

These findings suggest that aging of the PDL may be an important factor in the severity of periodontal disease through a higher production of inflammatory mediators in response to mechanical stress.

### Gingival Crevicular Fluid During Orthodontic Tooth Movement

Gingival crevicular fluid (GCF) is an osmotically mediated inflammatory exudate found in the gingival sulcus, where it tends to increase in volume with inflammation and capillary permeability. Serum is the primary source of the aqueous component of GCF; however, the gingival tissue through which the fluid passes, along with bacteria present in the tissue and gingival crevice, can modify its composition. Therefore, its constituents, which are derived from a variety of sources, including microbial dental plaque, host inflammatory cells, host tissue, and
serum, vary according to the condition of the periodontal tissues. In general, cells, immunoglobulins, microorganisms, toxins, and lysosomal enzymes can all be detected in GCF, while the mechanism of bone resorption might also be related to the release of inflammatory mediators present in GCF. Recently, a number of GCF constituents have been shown to be diagnostic markers of active tissue destruction in periodontal diseases, though only a few studies have focused on those involved in bone remodeling during orthodontic tooth movement. Mogi et al. found that GCF concentrations of IL-1β and IL-6 were significantly higher in a group with severe periodontal disease compared with controls, and Yavuzyilmaz et al. demonstrated the GCF IL-1β and TNF-α levels had a positive correlation to mean pocket depths, and suggested that those cytokines may be involved in the pathogenesis of periodontal diseases. Further, Mogi et al. reported that an increased concentration of RANKL and decreased concentration of OPG were detected in GCF from patients with periodontitis, while the ratio of RANKL concentration to that of OPG in GCF samples was significantly higher for periodontal disease patients than for healthy subjects. Taken together, these data suggest that RANKL and OPG contribute to osteoclastic bone destruction in periodontal disease.

Storey proposed that the early phase of tooth movement involves an acute inflammatory response characterized by periodontal vasodilation and migration of leukocytes out of the capillaries. Recent research has led to the hypothesis that inflammatory mediators are released following mechanical stimulus, triggering the biologic processes associated with alveolar bone resorption and apposition. Among the local biochemical mediators are cytokines, which are secreted by mononuclear cells and leukocytes. Cytokines can provoke the synthesis and secretion of numerous substances that form the molecular basis for cell-to-cell communication, including PGs and growth factors, thus interacting directly or indirectly with bone cells. Uematsu et al. found that the levels of inflammatory mediators (IL-1β, IL-6, TNF-α, epidermal growth factor, and β2 microglobulin) in GCF were elevated during orthodontic treatment, and Grieve et al. reported similar results for PGE and IL-1β. Further, Lowney et al. described an increase in TNF-α in GCF from teeth undergoing orthodontic forces. As noted above, inflammatory mediators have been detected in GCF samples during orthodontic tooth movement in the early phase. Our laboratory found an increased concentration of RANKL in GCF during orthodontic tooth movement, and the ratio of concentration of RANKL to that of OPG in the GCF was significantly higher than in control sites in another study. Consequently, analysis of GCF samples may provide a better understanding of the biochemical processes associated with tooth movement and can help clinicians make therapeutic choices based on qualitative and quantitative information.

PAIN DURING ORTHODONTIC TOOTH MOVEMENT

Vandevska-Radunovic summarized pain during orthodontic tooth movement as follows. Pain is one of the cardinal signs of inflammation, as well as a nearly inevitable and, for the patient, most unpleasant reaction to orthodontic therapy. Furstman and Bernick suggested that periodontal pain is caused by a process of pressure, ischemia, inflammation, and edema. Burstone identified both immediate and delayed pain responses, which begin a few hours after application of an orthodontic force and then last for approximately 5 days. Pain results, in part, from the stretching and distortion of tissues due to mechanical forces, as well as from interactions of multiple inflammatory mediators with local pain receptors. PGs have been shown to cause hyperalgesia, which is an increased sensitivity to noxious agents such as histamine, bradykinin, serotonin, acetylcholine, and substance P. There are indications that perceptions of pain in the PDL are due to changes in blood flow, though they can cause gastrointestinal ulceration, renal injury, and disruption of platelet function and hemostasis.

ANT-INFLAMMATORY EFFECTS OF LASER IRRADIATION

The use of modulating agents, including antiproteinase to inhibit matrix metalloproteinases, anti-inflammatory drugs to block the production of pro-inflammatory cytokines and PGs, and bone-sparing agents to inhibit activation of osteoclasts, has been postulated to be of therapeutic value as adjunctive therapy for the management of periodontitis. Non-steroidal anti-inflammatory drugs (NSAIDs) and bisphosphonate drugs may also have an adjunctive role in periodontal therapy, as NSAIDs have been shown to have a role in postoperative pain management, though they can cause gastrointestinal ulceration, renal injury, and disruption of platelet function and hemostasis.
Various bio-stimulatory effects of low-energy laser irradiation have been reported, including wound healing\textsuperscript{53, 78}, fibroblast proliferation\textsuperscript{8, 111, 121}, chondral proliferation\textsuperscript{102}, collagen synthesis\textsuperscript{1, 7}, and nerve regeneration\textsuperscript{3}. Some anti-inflammatory effects of low-energy laser irradiation have been shown \textit{in vivo}, including the inhibition of carrageenin inflammation in rats\textsuperscript{46, 47}, inflammatory cellular infiltration in the synovial membrane of patients with rheumatoid arthritis\textsuperscript{2}, and PGE\textsubscript{2} in the synovial fluid of a patient with rheumatoid arthritis\textsuperscript{12}. A clinical investigation showed that low-level laser therapy reduced orthodontic post-adjustment pain\textsuperscript{67}, and we reported that low-energy laser irradiation inhibited the incidence of open gingival embrasure space following orthodontic treatment\textsuperscript{77} and that PGE\textsubscript{2} and IL-1\textbeta production in stretched human PDL cells was inhibited by laser irradiation \textit{in vitro}\textsuperscript{107}. Further, a recent study showed that low-energy laser irradiation accelerated orthodontic tooth movement\textsuperscript{20}. Therefore, low-energy laser irradiation may be useful in inhibiting inflammation and pain as well as decreasing orthodontic treatment time without side effects (Fig. 4).

\section*{CONCLUSIONS}

In summary, mechanical stimulus causes an inflammatory reaction within periodontal tissues, which in turn may trigger the biological processes associated with bone remodeling. Further, low-energy laser irradiation may be useful in inhibiting inflammation and stimulating bone remodeling.

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