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Chapter 5

Advances in Orthodontic Tooth Movement: Gene Therapy and Molecular Biology Aspect

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Abstract

Accelerated orthodontic tooth movement has been recently the topic of interest for orthodontic practitioners. Increased numbers of both clinical and research articles associated with the accelerated orthodontic treatment have been published in peer-reviewed journals in the last couple of years. Biochemical approaches such as administration of drugs, vitamins, and proteins and/or physical approaches such as surgery, vibration, and photobiomodulation have been widely reported and demonstrated the predicted outcome; however, the results are controversial. Very few reports addressed on genetic background of patients or utilization of molecular biological approach on the accelerated orthodontic treatment. In this chapter, we will discuss about biology of tooth movement and how the advances in gene therapy and molecular biology technology would shape the future of orthodontic treatment.

Keywords: gene therapy, molecular biology, orthodontic, accelerated tooth movement

1. Introduction

Orthodontic tooth movement is a biological process that requires the relay of mechanical loading to biological signals by periodontal ligament (PDL) and alveolar bone (AB) cells such as osteoblasts, osteocytes and osteoclasts. The mechanotransduction of signals involves dynamic cellular communication which allows for coordinated cellular response of alveolar bone remodeling and periodontal tissue homeostasis that occurs in response to orthodontic force. This complex process depends on adaptive tissue remodeling of periodontium for both anabolic and catabolic events. Compression and tension forces from orthodontic treatment create stress and strain to the PDL and AB cells and their surrounding extracellular matrices (ECM), which respond to the stress and strain from orthodontic forces by expressing and secreting

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biologic mediators and inflammatory cytokines, osteoclast differentiation factors and ECM proteins such as collagen I, III, V and their modifying enzymes and proteases. These biomolecules, in turn, initiate the activation of fibroblasts, osteoblasts, osteocytes and recruitment and differentiation of osteoclasts leading to anabolic activities on the tension side and increased osteoclastic activity and low bone density on the compression side of tooth movement. These cellular and molecular events are strictly controlled at transcriptional, posttranscriptional and translational levels and the interference of these events affects the rate of tooth movement. Therefore, understanding the mechanism of cellular and molecular events of tooth movement will allow us to apply the cutting edge knowledge to improve clinical orthodontic practice using gene therapy or molecular biology approaches.

2. Orthodontic tooth movement models

Several models have been proposed for mechanism of initiation of orthodontic tooth movement as below.

1. Pressure-tension model: it was derived from the observation of experiments from animal models, in which a force of a given direction was applied to a tooth to create the tension and compression areas in periodontal tissues [1–4]. The histological studies demonstrated that bone was deposited on the alveolar wall on the tension side of the tooth in the presence of both heavy and light forces, with newly formed bone spicules followed the orientation of the periodontal fiber bundles. On the compression side, with the light forces, alveolar bone was resorbed directly by numerous multinucleated osteoclasts in Howship's lacunae (frontal resorption). In contrast, the periodontal tissues were compressed with heavy forces, leading to capillary thrombosis, cell death and the production of localized cell-free areas (hyalinization). Hyalinization phenomenon was later supported by several investigators [5–7]. At the hyalinization sites, osteoclastic resorption of the adjacent alveolar wall did not take place directly, but was initiated from the neighboring marrow spaces referred as 'undermining resorption' [8].
2. Bone bending/piezoelectric current model: it was observed that the deformation that occurred when an external load was applied to a long bone produced electrical current in the surface curvature of the bone. Increased bone concavity was shown to be associated with electronegativity and bone formation; while increased bone convexity was associated with electropositivity and bone resorption [9]. This model has major flaws given the fact that piezoelectricity does not require the presence of living cells. Dead bone produces the same effects, which appear to be generated by shearing forces acting on the collagen fibers of the bone matrix. Therefore, the stress-generated electrical potentials could be a by-product of deformation. In addition, the magnitude of the current is small and may not be sufficient to induce cellular changes [8, 10].
3. Neurogenic inflammation model: it was based on the assumption that orthodontic tooth movement was the result of inflammatory processes triggered by peripheral nerve fibers referred as neurogenic inflammation. This inflammation is characterized by the release of neuropeptides such as calcitonin gene-related peptide (CGRP) and substance P upon the stimulation of

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afferent nerve endings [11]. A report showed that the nerve ending released the neuropeptides after periodontal ligament had been strained by the force applied to the tooth [12].

4. Fluid flow shear stress model: it was based on the concept that osteocytes respond to mechanical forces. Locally strain derived from the displacement of fluid in bony canaliculi of osteocytes is very important [13]. When loading occurs, interstitial fluid squeezes through the thin layer of the non-mineralized matrix surrounding the cell bodies and cell processes, resulting in local strain at the cell membrane and activation of the affected osteocytes [14]. With regard to orthodontic force, the force on the side of the tooth receiving orthodontic pressure creates shear stress and activates responses on osteocytes [15]. The shear stress on the osteocytes induces increased secretion of biological mediators from the osteocytes leading to activation of osteoclasts [16, 17]. At the same time, on the tension side, the increased pulling force on the periodontal ligament is transferred to the bone. The resulting deformation drives the fluid flow shear stress on the network of osteocytes. This shear stress induces osteocyte activation, and osteocytes respond by secreting signaling molecules that contribute to osteoclast recruitment and differentiation.

In addition, it has been shown that compressive force induces bone matrix deformation and microcracks; and the accumulation of microscopic cracks in the bone matrix may induce additional damage to osteocytes in the microcrack region [18]. Microcracks are more prevalent on the pressure-side than on the tension-side of the tooth, and it has been hypothesized that microcracks were the first damage induced by the orthodontic force to induce osteocyte apoptosis and bone remodeling. Osteocyte apoptosis has been observed at the pressure side in an experimental tooth movement model in animal models, which may be associated with the subsequent bone resorption [19, 20]. Therefore, the microcracks may play a role in the initiation of bone resorption on the pressure side of the tooth under the compressive force of orthodontic loading [21].

3. Molecular mechanism of orthodontic tooth movement

Although there are several models proposed to explain the events of orthodontic tooth movement, no single model could directly and comprehensively explain this process. The evidence from histological and animal studies has shown that this complex biological process is initiated from the application of mechanical forces onto the orthodontic appliances, which converts into the biological signals to stimulate mechanosensitive cells. (**Figure 1**) [22] Literatures showed that orthodontic force application induced physiologic adaptation of alveolar bone with small magnitude of reversible injury to periodontium [23]. Significant evidence suggests that when mechanical loading forces are relayed from the orthodontic appliances to the PDL and bone tissues, the mechanoreceptor cells percept the loading forces as shear stress and strain [24] as the tooth shifts its position in the PDL space resulting in compression and tension areas in PDL and bone tissues [25].

The sequence of biological events after loading of orthodontic force occurs as (1) fluid flow changes and matrix strain (**Figure 2**); (2) strain on mechanoreceptor cells (**Figure 2**); (3) cell activation (**Figure 3**); and (4) tissue remodeling leading to tooth movement (**Figure 4**) [15].

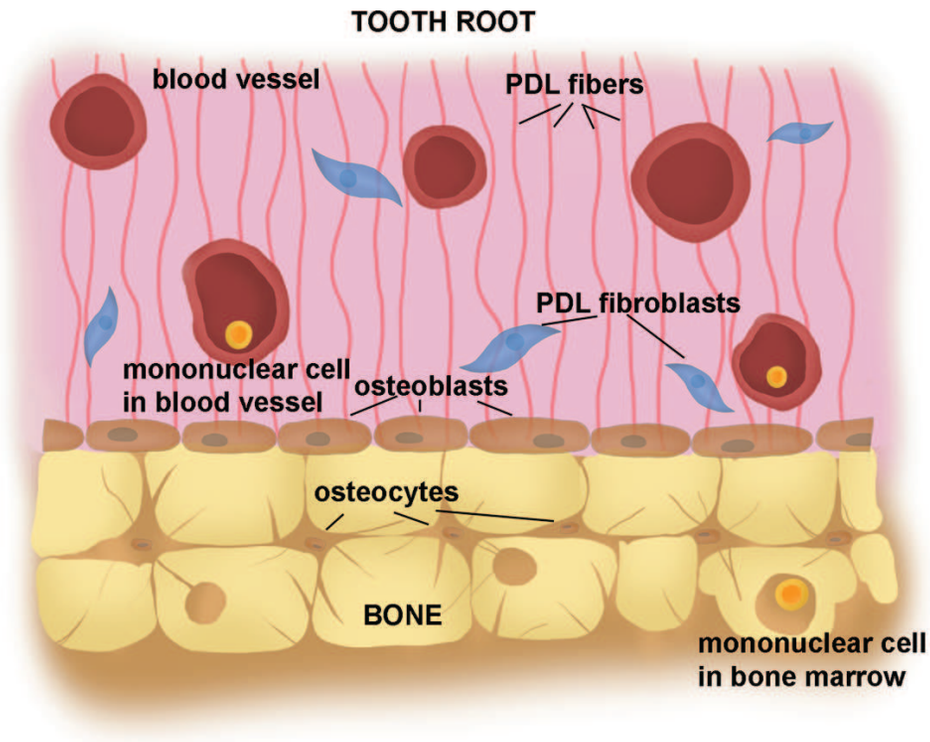


Figure 1. Illustration of cellular events of periodontal ligament and alveolar bone at non-loading state. Blood vessels and periodontal fibroblasts reside in between the periodontal ligament collagen fibers. Inactive osteoblasts line along the alveolar bone surface and quiescent osteocytes reside in their bony lacunae. Modified from Hatch [25].

The mechanoreceptor cells in periodontal tissue include osteocytes and bone lining osteoblasts in alveolar bone and fibroblasts in PDL. The final result as tissue remodeling occurs in both mineralized and non-mineralized ECM during the tooth movement [26]. Recent studies have indicated that osteocytes are capable of sensing strain in their bone lacunae following mechanical loading of the bone [21]. The mechanism of how osteocytes sense, transfer, and respond to mechanical strain remains unclear. Osteocyte processes have been shown to utilize integrins, gap junctions and ion channels to respond to mechanosensing external physical stimuli [27, 28]. Fluid flow-induced shear stress is the strain resulted from an immediate change in fluid flow in the lacunar-canalicular system leading to an increasing strain on the osteocytes. This shear stress can amplify the mechanical signals to the osteocytes [14, 29]. Several proteins such as integrins, connexin 43, osteopontin, and vitronectin, and several transcriptional factors such as c-Fos expression in the osteocytes are affected by loading forces [30–32]. In addition, the reduced number of primary cilia of osteocytes could affect their secretion of prostaglandins (PGs) and increased cyclooxygenase-2 (COX2) and RANKL/OPG ratio in osteocytes in response to fluid flow shear stress [33, 34]. Recent studies showed that osteocytes can induce both anabolic and catabolic bone signals in response to loading [35–37], yet the mechanism of how osteocytes switch from catabolic activity to anabolic activity is unclear. Under compression, osteocytes undergo apoptosis and are coupled with bone resorption [19, 38]. However, fluid flow shear stress may induce osteocytes to secrete anabolic bone proteins such as prostaglandin-E2 (PGE2) or nitric oxide (NO) [39, 40]. Several recent evidence demonstrated the significance of osteocytes during osteoclast differentiation and

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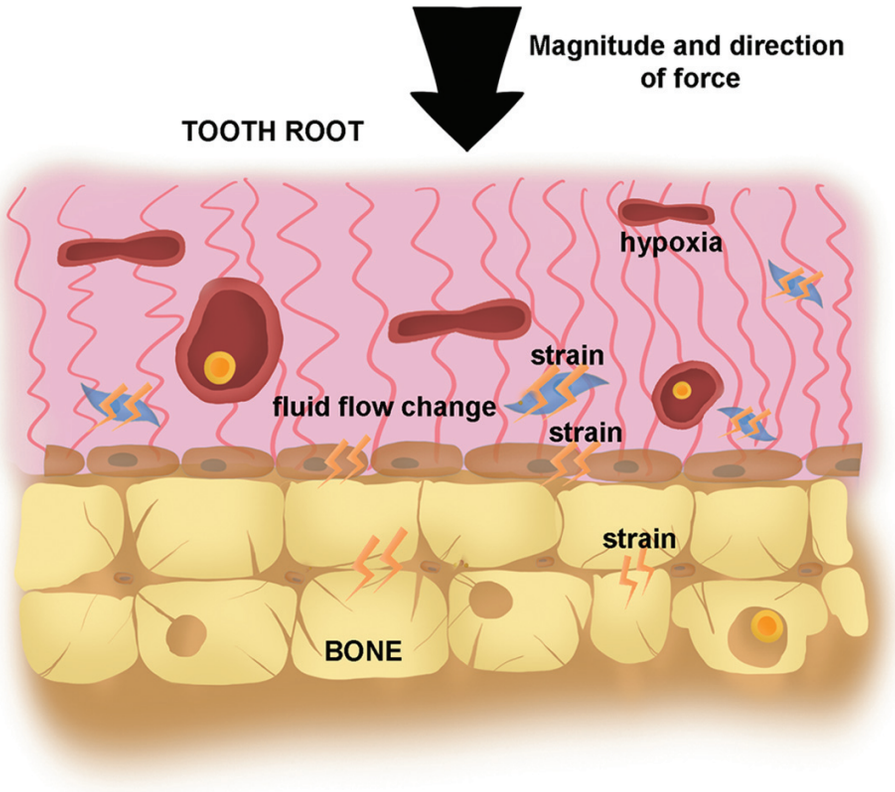


Figure 2. Initial cellular events in periodontal ligament after force loading during tooth movement. The blood vessels are squeezed then local hypoxia and fluid flow change are initiated from the loading force. The mechanical strain affects the periodontal fibroblasts and osteoblasts in the periodontal ligament space. The strain creates fluid flow shear stress and strain on the osteocytes in their bone lacunae. The mechanical strain induces secretion of inflammatory cytokines and biological signaling mediators including interleukins, prostaglandins, tumor necrosis factors, nitric oxide, growth factors, proteinases and cell differentiation factors. These mediators, in turn, activate the periodontal fibroblasts, osteoblasts and osteocytes. Modified from Hatch [25].

activation [41–43]. The osteocyte ablation *in vivo* caused a significant reduction in osteoclastogenesis and osteoclastic activity under loading forces, suggesting the important roles of osteocytes during orthodontic tooth movement [44]. Increased evidence supported the close association between osteocytes and osteoclasts during tooth movement. Experimental tooth movement in mice demonstrated increased expression of osteopontin [45], matrix extracellular phosphoglycoprotein (MEPE) [46], and receptor activator of nuclear factor-kB ligand (RANKL) [43, 47] in osteocytes. These proteins play important roles in osteoclastic activity and osteoclastogenesis because deficiency of these proteins results in significant reduction or absence of the osteoclasts and increased bone mass in the animals [43, 48]. Osteocyte apoptosis occurred abundantly on the compression side of tooth movement in 1 day after loading, and then an increased number of osteoclasts were observed until day 3, resulting increased tooth movement by day 10 [49]. It is speculated that apoptotic osteocytes may release signaling proteins such as RANKL and interleukin (IL), to osteoclast precursors, and initiate osteoclastogenesis. In contrast, when subjected to fluid flow sheer stress, osteocytes secrete NO and PGE2, which these proteins have potent, anabolic, and direct effects on osteoblasts [40, 50, 51]. PGE2 expression increased in loaded bone tissue [52]. NO secreted from osteocytes promotes osteoblast differentiation, and

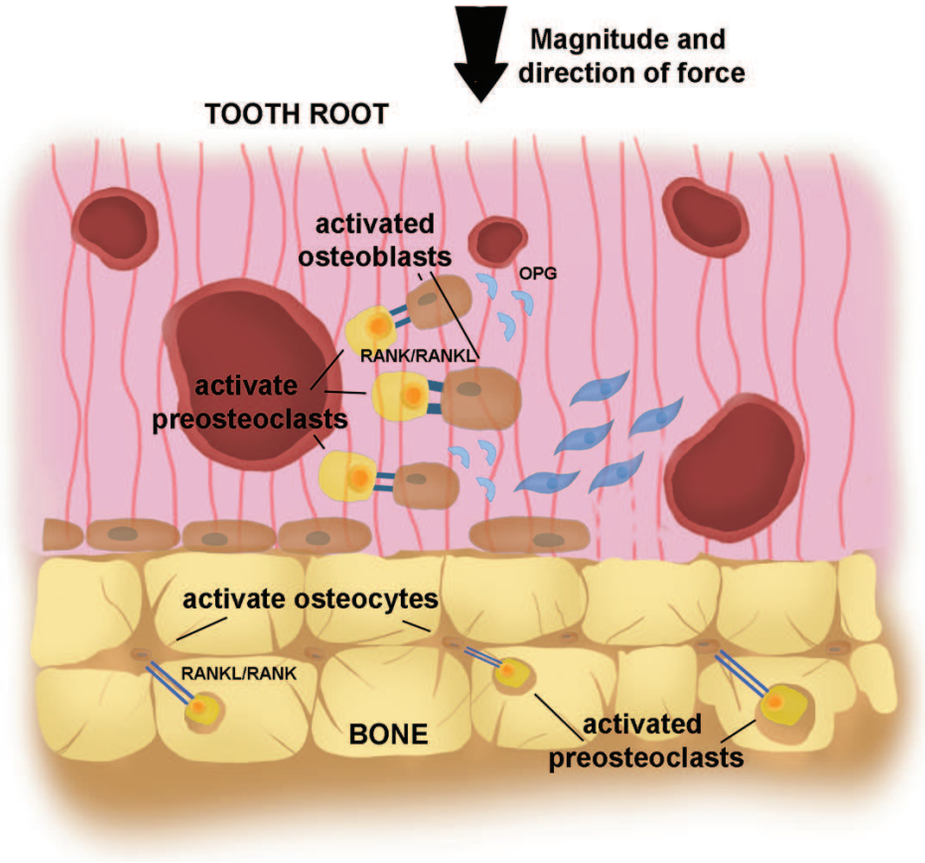


Figure 3. Intermediate cellular events in periodontal ligament during tooth movement. The blood vessels dilate due to the response to the released mediators and cytokines. The activated fibroblasts, osteoblasts and osteocytes are ready to secrete M-CSF and RANKL to activate preosteoclasts from blood and bone marrow. In addition, the activated osteoblasts release OPG to act as competitive decoys for RANKL. The PDL fibroblasts release MMPs to degrade collagen fibers in the periodontal ligaments. Modified from Hatch [25].

plays an important role in bone formation during loading [40, 53]. NO can influence bone mass and simultaneously inhibit osteoclast activity [54]. Increased NO production by osteocytes after mechanical stimulation by fluid flow modulates apoptosis-related gene expression suggesting that NO maintains osteocyte viability [55].

Beside osteocytes, preosteoblasts are also responsive to mechanical force. Mechanical force loading triggers several cell signaling pathways in osteoblasts such as calcium (Ca^{2+}), NO, IL1β and adenosine triphosphate (ATP) in a short period of time [24, 56, 57]. NO and IL are potent mediators secreted during orthodontic tooth movement [58, 59]. Preosteoblast differentiation can be induced on the tension side of tooth movement via integrin/focal adhesion kinase signaling and Ca^{2+} channels [60, 61]. Fluid shear stress can trigger Ca^{2+} signaling pathway and promotes ATP release, PGE2 secretion and proliferation of osteoblasts [24, 57]. While on the compression side, reduced blood flow in PDL and localized hypoxia occurs. The reduction in O_2 tension stabilizes hypoxia inducible factor-1 (HIF-1), a transcription factor that activates vascular endothelial growth factor (VEGF) and RANKL expression in PDL fibroblasts and osteoblasts leading to osteoclast differentiation and favoring bone resorption in areas of compression [62–64]. As mentioned above, inflammatory cascade is important for orthodontic

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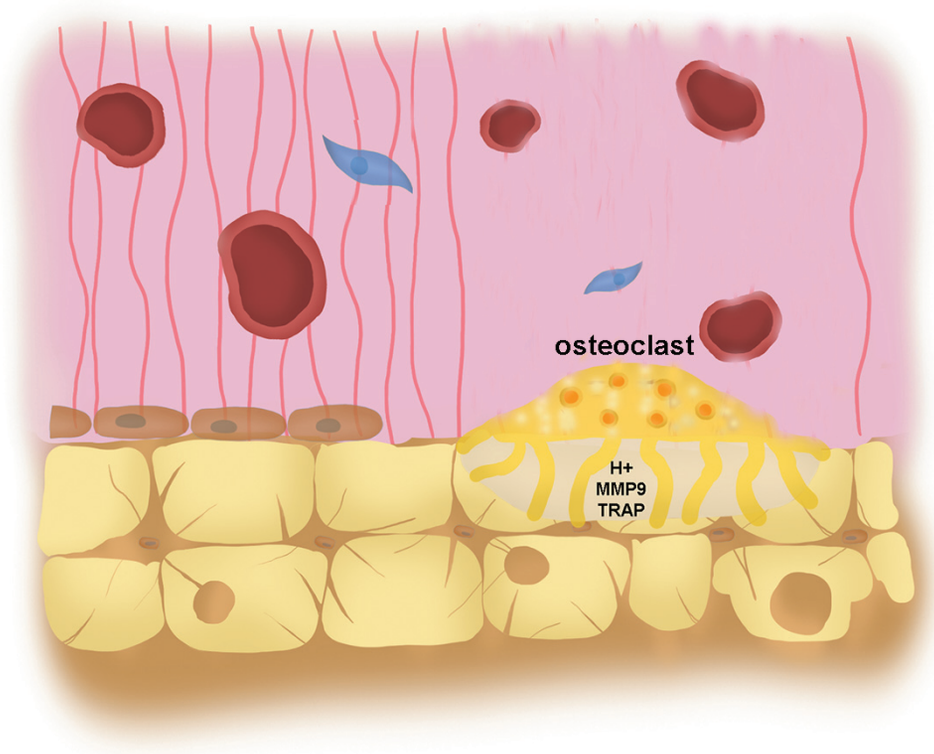


Figure 4. Late cellular events in periodontal ligament and alveolar bone front during tooth movement. The activated osteoclast is derived from the fusion of preosteoclasts, creates ruffle border to seal the bone surface area and releases MMP9, TRAP and acid to resorb bone matrix and minerals. Apoptotic osteocytes also release the biomolecules and mediators to activate osteoclast recruitment for bone resorption leading to tooth movement. Modified from Hatch [25].

tooth movement. During the process, inflammatory cytokine such as IL-1β, PGE2, tumor necrosis factor-alpha (TNF-α) and NO are secreted from preosteoblast in PDL and osteocytes in bone lacunae during the orthodontic tooth movement [59, 65, 66]. Compression is associated with elevated COX-2 which catalyzes production of PG, including PGE2, from arachidonic acid [67, 68]. Administration of PGE2 into alveolar bone of mice induces both osteoclasts and osteoblasts [26]. During orthodontic tooth movement, pain sensation occurs and, coincidentally, substance P and calcitonin gene related peptide (CGRP) are induced to be secreted during the tooth movement. These neuropeptides can enhance cellular secretion of inflammatory cytokine and in turn increase vasodilation and permeability of surrounding blood vessels [69–72]. Several evidence showed that the inhibition of inflammation hindered tooth movement [73, 74], while inflammation in the alveolar bone promoted tooth movement [75, 76].

Osteoclasts are the major key cells that play significant roles during tooth movement. Osteoclasts are multinucleated giant cells which are formed by the fusion of mononucleated osteoclast precursors derived from hematopoietic origin and function to resorb the alveolar bone during tooth movement. The osteoclast progenitor cells require macrophage colony stimulating factors (M-CSF) for their proliferation and survival. M-CSF is a secreted cytokine by osteoblasts and affects osteoclast progenitors. The RANK/RANKL/OPG system has been a crucial mechanism in osteoclastogenesis during bone resorption and tooth movement [77–79]. Receptor activator for nuclear factor κB (RANK) is a transmembrane protein and a member of tumor necrosis factor

receptor family that is expressed on osteoclastic precursors, preosteoclasts and osteoclasts. Receptor activator for nuclear factor κB ligand (RANKL) is a transmembrane protein and is a member of the tumor necrosis factor superfamily that is expressed on preosteoblasts, osteoblasts and osteocytes [80]. RANK is the receptor for RANKL and the binding between both of them stimulates the differentiation of preosteoclasts into mature osteoclasts. Osteoprotegerin (OPG) is a soluble extracellular tumor necrosis receptor protein that is secreted by preosteoblasts and osteoblasts. OPG is a decoy receptor for RANKL in regulating bone metabolism and inhibiting osteoclastogenesis and bone resorption. RANKL/OPG ratio is an important determinant of bone mass and skeletal integrity and also an indicator for the osteoclast function [78, 79]. Increased evidence demonstrated the direct association of tooth movement and activities of osteoclasts. Accelerated osteoclast resorption in alveolar bone of OPG deficient mice was observed during tooth movement [81] while inhibition of RANKL or deletion of RANKL in mice resulted in suppression of tooth movement [47]. In addition, local administration of M-CSF resulted in modulation of rate of tooth movement in animals [82].

Overall, the mechanism of tooth movement is complex and need strictly coordinated regulation of PDL, osteoclasts, osteocytes and osteoblasts. It is very challenging clinically to apply optimal force onto the tooth to avoid hyalinization. Clinically, tooth movement in patients is a result of combination of undermining and frontal resorption [83]. Compression sides involve increased expression of PGE2, TNF-α and IL-1β. PGE2 promotes osteoblast and osteoclast differentiation and activity. Activated osteoblasts secrete RANKL and OPG to trigger osteoclast differentiation and activity. TNF-α and IL-1β promote osteoclast differentiation and activity. In addition, matrix metalloproteinases (MMPs) expression is increased as well as the expression of M-CSF [84]. Loading compressive force affects osteocytes to upregulate the expression of connexin 43 [85], endothelial nitric oxide synthase (iNOS) [50], osteopontin [45], SOST [86] and RANKL [47]. These molecules recruit osteoclast precursors and activate osteoclasts to resorb the alveolar bone on the compression side. While on the tension side, increased expression of transforming growth factor-β (TGF-β), a potent ECM growth factor, was detected [87]. Several anabolic molecules such as bone sialoprotein (BSP) [88], collagen I (ColI) [89], vascular endothelial growth factor (VEGF) [84, 90], tissue inhibitors of metalloproteinases (TIMPs) [91], insulin-like growth factor (IGF) and its related receptor [92], heat shock protein 27 (HSP 27) [93] and ATP [94] were increasingly expressed on tension side during tooth movement. IL-6 around the osteocytes under loading can promote its signaling toward osteoblast pathway [53]. The presence of TIMPs around tension side is speculated to control the activity of MMP and remodeling pattern in alveolar bone. The anabolic events such as increased osteoblast activity and decreased osteoclast activity occur on the tension side of tooth movement.

4. Studies on genetic manipulation of tooth movement

Administration of proteins that affect or activate osteoclasts could be a direct approach to modulate tooth movement though the dosage and side effects such as root resorption are factors of consideration. With modern advanced technology, the manufacturer can generate a large amount of human recombinant proteins for therapeutic purposes. However, the life span

of these proteins once administered in human body is short and may not reach therapeutic level [95]. Gene therapy is a therapeutic approach that uses genes to treat or prevent diseases. Gene therapy is designed to introduce nucleotides into the cells to compensate for mutated genes or to restore the normal protein. If a mutation causes a crucial protein to be defective or missing, gene therapy may be able to introduce a normal copy of the gene to restore the function of the protein. After integration of the genes that encoded the target protein into the patient's genetic machinery, gene therapy can allow the body to produce the required protein constantly so the level of protein will be constantly high at therapeutic level [96]. The concept of gene therapy includes cloning of selected DNA/RNA fragments into a delivery system in order to administer into the host or patient. The delivery system could be viral vectors or non-viral vectors such as liposomes, peptides, polymer particles, gene gun and electric perforation [97]. The clinical application of gene therapy can be achieved with *in vivo* or *ex vivo* approaches. The *in vivo* gene therapy will include injection of vectors into the patient directly while the *ex vivo* approach includes the introduction of vector into the cells then the transfected cells are transplanted back into the patient [98–100].

Recently gene therapy has been approved to be implemented in medicine. The U.S. Food and Drug Administration (FDA) regulates all gene therapy products in the United States and oversees research in this area. In medicine, the FDA recently approved gene therapy for the treatment of some types of leukemia and inherited blindness [101]. Several experiments of gene therapy in dentistry involved orofacial pain, squamous cell carcinoma, tooth and bone regeneration, salivary gland disease and orthodontic treatment [102].

The gene therapy experiments in orthodontic treatment are still limited to cell cultures or animal experiments [103]. The purposes of previous gene therapy in orthodontic treatment were to investigate the possibility of acceleration of tooth movement or reduction of root resorption by modification of osteoclast differentiation factors such as RANKL or OPG [104–109]. The first attempt for gene therapy in orthodontic treatment aimed to transfer OPG gene into periodontal tissue to reduce osteoclast activity and inhibit tooth movement. The gene transfer approach using a hemagglutinating virus of Japan (HVJ) envelope vector carrying mouse OPG messenger RNA (mRNA) was performed in rats for 21 days of tooth movement. The vector solution was administered into rat's palatal gingiva by infiltration injection. The result showed that local OPG gene transfer reduced the number of osteoclasts and decreased tooth movement by 50% in rats in the experimental group compared to the ones in the control group. The effect of OPG gene transfer was local and did not affect bone mineral density of tibia of the animals [105]. The same group of investigators performed another experiment using the same system to transfer mouse RANKL mRNA to periodontal tissue to activate osteoclastogenesis and accelerate tooth movement in rats. The results showed that local RANKL gene transfer induced increased numbers of osteoclasts and accelerated tooth movement by approximately 150% in the rats in the experimental group compared to the control group. The effect of RANKL gene transfer was local and did not elicit any systemic effects. Interestingly, the number of osteoclasts was reduced time dependently after gene transfer [104]. Another group of investigators compared corticotomy with gene therapy using a hemagglutinating virus of Japan envelope vector containing mouse RANKL mRNA in rats for 32 days. The results showed increased level of RANKL protein 3 folds in the gene therapy group and 2 folds in the corticotomy group after 10 days; however, the level

of RANKL protein was maintained in the gene therapy group but not in the corticotomy group. The number of osteoclasts in the RANKL gene therapy group was significantly higher at day 10 with or without tooth movement compared to the tooth movement only group. The tooth movement distance was 2 times more in the RANKL gene therapy group and 1.5 times in the corticotomy group; however, the rate of tooth movement slowed down in the corticotomy and controls groups but was constant in the RANKL gene therapy group. It was concluded that gene therapy was an alternative treatment for corticotomy to accelerate tooth movement and the efficacy of treatment was higher than corticotomy to accelerate tooth movement [106]. The OPG gene transfer experiment was performed by another group of investigators using the same viral envelope packaging and delivery system to investigate the inhibition of orthodontic relapse in rats. The first molars in the rats were moved mesially for 3 weeks then the springs were removed to generate orthodontic relapse in the rats. The rats received OPG gene therapy then were observed for 2 weeks. The results showed that relapse was significantly inhibited 2 times compared to the mock and control groups. The bone mineral density and bone volume fraction of alveolar bone were significantly increased in the gene therapy group compared to the mock and control groups. No difference of bone mineral density and bone volume fraction of tibia was found among groups. The investigators stated that local OPG gene therapy to periodontal tissues could inhibit relapse after orthodontic tooth movement via osteoclastogenesis inhibition [110]. The same group of investigators further investigated the effect of local OPG gene therapy on orthodontic root resorption with the same design of experiment. They utilized a microcomputed tomogram and histological analyses. The result showed no difference between root resorption at the beginning and the end of tooth movement in the OPG gene therapy group. However, the repair of root resorption in the gene therapy group was higher than other control groups [107]. Another study investigated the effect of local OPG gene therapy using mesenchymal stem cells as carriers for plasmid containing OPG mRNA. This cell mediated OPG gene transfer was generated by insertion of plasmid containing OPG mRNA into the mesenchymal stem cells and the cells were injected into the animals. The result showed that the cells containing OPG package grew in the animals' PDL and the number of osteoclasts, level of RANKL and bone resorption were reduced significantly after single injection. The level of OPG was highest in the gene therapy group [108].

Gene therapy is a promising treatment option for a number of diseases (including inherited disorders, some types of cancer, and certain viral infections). This approach is still in the developing process as an alternative approach to treat deformity or disease that conventional method could not achieved. Although many clinical trials have shown the efficacy of the treatment, the technique remains risky and is still under processes of investigation to make sure that it will be safe and do not elicit any systemic or hereditary effects for the patients.

5. Future of genetic manipulation of tooth movement

With the rise of advanced technology in biomedical engineering and medicine, gene therapy is no longer a science fiction. Several gene therapies have been approved to treat many conditions and deformities not only in the United States but worldwide [111]. In the past decade, gene

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targeting using endogenous microRNA (miRNA) has emerged as a powerful tool for targeted gene delivery. miRNAs are short, noncoding and highly conserved RNA sequences that tightly regulate the expression of genes by binding to their target sequence in the corresponding mRNAs [112, 113]. Majority of miRNA biogenesis involves transcription by RNA polymerase II to generate primary microRNA (pri-miRNA) followed by Drosha (RNase III enzyme) processing, which produces precursor miRNA (pre-miRNA). The pre-miRNA is transported to the cytoplasm via exportins/RanGTP complex. In the cytoplasm, the pre-miRNA is cleaved by another RNase III enzyme called Dicer to generate mature miRNA. The mature miRNA then forms a microRNA associated RNA-induced silencing complex (miRISC) with Argonaute proteins. The complex is steered to the target mRNA via base pairing with the target sequence of the miRNA. The degree of perfect complementarity at nucleotides 2–8 (binding sequence) in the 5′-end of the miRNA is essential for a successful action of the RISC complex. Depending on the extent of complementarity with the target sequence, gene expression is repressed either by inhibition of translation or by cleavage of the corresponding mRNA [114]. The process of gene therapy using endogenous miRNAs involves selection process of miRNA candidates, design of expression cassettes if constant expression is needed, selection of delivery carrier, and evaluation of system in cells, animal models and clinical trials [114]. Several miRNAs have been reported for their expression and roles in PDL and alveolar bones [115–118]. Under loading, several miRNAs in PDL and alveolar bone respond to the loading force and orientation of forces in different pattern of expression [119–121]. miRNA-21 has been shown to have critical roles in PDL, osteoblasts and osteoclasts [120, 122–127]. In addition, miRNA-21 deficient mouse demonstrated delayed tooth movement compared to the control mice via inhibition of osteoclastogenesis [127]. miRNA-29 was reported as a crucial miRNA for alveolar bone remodeling during tooth movement due to its expression under different orientation of loading forces and its expression profile in crevicular fluid during tooth movement in human [121, 128]. miRNA-29 expression in human PDL was up-regulated under compression but down-regulated under stretch force orientation [121] and its expression on crevicular fluid increased along the course of tooth movement [128]. Moreover, miRNA-29 sponge transgenic mice demonstrated delayed tooth movement due to the decreased numbers of osteoclasts [129]. These microRNAs could be a target candidate for gene therapy for orthodontic tooth movement. There are viral and nonviral delivery systems in clinical trials for gene therapy. Among viral vector system, lentiviral vector-based system has been developed and tested for its safety for more than 10 years. Non-integrating lentiviral vector have been investigated as a means of avoiding insertional mutagenesis. However, there is a disadvantage of this approach regarding the short-lived of the vectors in dividing cells [130]. Nonviral gene delivery systems (nVGDS) have great potential for therapeutic purposes and have several advantages over viral delivery including lower immunogenicity and toxicity, better cell specificity, better modifiability, and higher productivity. However, there is no ideal nVGDS; hence, there is widespread research to improve their properties [97]. The nVGDS system includes chemicals, peptides, liposomes, and polymers [97]. Exosomes are small (30–150 nm in diameter) extracellular vesicles that are formed in multivesicular bodies and are released from cells as the multivesicular bodies fuse with the plasma membrane. The exosomes were proposed to be used for delivery of miRNAs, protein and oligonucleotide complex [131], and were found to be cell secreted from osteoclasts [131] and in gingival crevicular fluid during the course of tooth movement [128].

Another genome editing system that has recently gained attention in research and clinical application is CRISPR/Cas9 system. The CRISPR/Cas9 system is based on CRISPR (clustered regularly interspaced short palindromic repeats) sequence and CRISPR associated (Cas) gene mechanism that are crucial for innate defense mechanism in bacteria and archaea enabling the organisms to respond to and eliminate invading genetic materials from their phages [132]. The CRISPR/Cas9 system consists of two key molecules that introduce a mutation into the DNA. First, Cas9 is an enzyme that acts as a pair of DNA scissor. It cuts the two strands of DNA at a specific location in the genome so the genome editing could be performed either addition or removal. The other molecule is guide RNA (gRNA) which consists of a small piece of predesigned RNA sequence (~20 bases long) located within a long RNA scaffold. The long RNA scaffold binds to DNA and the gRNA sequence guides Cas9 to edit the specific part of genome. gRNA sequence is designed to be complementary to the target DNA sequence in the target gene in the genome. gRNA sequence consists of short palindromic repeats and the sequences that complement with the target genes. The target sequences should be present close to protospacer adjacent motif (PAM) sequence which increases the specificity of Cas9. After Cas9 nuclease enzyme site specifically cleaves double stranded DNA activating double-strand break repair machinery. If the DNA repair template is provided, the piece of DNA repair template will be inserted into the sequence of target genes [133, 134]. With this mechanism, the plasmid containing gRNA, Cas9 sequences, TracrRNA (transactivating CRISP RNA) and DNA repair template sequence can be introduced into cells or embryo of the animals by viral or nonviral delivery system [135]. Until now, there is no CRISPR/Cas9 experiment involving orthodontic tooth movement, however, this technology has been implemented in recent mineralized tissue research [136–139]. Future directions of gene therapy include the enhancement of the lentiviral vector-based approaches, fine tuning of the conditioning regimen, and the design of safer vectors or nonviral vector delivery system. In orthodontic field, the gene therapy approach will need several fundamental cell culture and animal experiments to demonstrate the safety and efficacy of the treatment concept. Clinical trials are required as the next step to ascertain the clinicians and patients for efficacy of the treatments.

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Conflict of interest

The authors declare no conflict of interest.

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