Babol University Of Medical Sciences

IJMCM, Autumn 2025, VOL 14, NO 4

International Journal of Molecular and Cellular Medicine

Journal homepage: www.ijmcmed.org

REVIEW ARTICLE

Regulation of Osteoblast Differentiation by miR-214 and miR-206 Through EphrinA2 Signaling: Emerging Therapeutic Insights in Orthodontic Tooth Movement

Hossein Salehivaziri[1], Amir Ali Didar[2], Maryam Sobhani[3], Ali Narimani [4], Moslem Karimzadeh[5*], Yasaman Bathaei[3]

1. Department of Periodontology, School of Dentistry, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
2. Department of Endodontics, School of Dentistry, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
3. Department of Orthodontics, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran.
4. Department of Prosthodontics, Faculty of Dentistry, University of Medical Sciences, Tabriz, Iran.
5. Islamic Azad University Tehran Medical Sciences, Tehran, Iran.

ARTICLE INFO

Received: 2025/07/9
Revised: 2025/08/30
Accepted: 2025/09/3

***Corresponding**:
Moslem Karimzadeh
Address:
Islamic Azad University Tehran Medical Sciences, Tehran, Iran.
E-mail:
moslem.k90@gmail.com

ABSTRACT

Orthodontic tooth movement (OTM) is a complex biological process involving the precise remodeling of alveolar bone in response to mechanical forces. This remodeling is mediated by the coordinated activities of osteoblasts (responsible for bone formation) and osteoclasts (responsible for bone resorption) within the periodontal ligament (PDL). Mechanical stimuli are transduced into biochemical signals, which regulate cellular behavior through key signaling pathways such as RANKL/RANK/OPG and Wnt/β-catenin.

MicroRNAs (miRNAs), as crucial post-transcriptional regulators of gene expression, play significant roles in skeletal development and bone homeostasis. They influence essential signaling cascades that govern the differentiation, function, and survival of osteoblasts and osteoclasts. Among them, miR-214 and miR-206 have emerged as potent negative regulators of osteoblast differentiation. This review focuses on how their common target, EphrinA2, plays a pivotal role in bone remodeling. EphrinA2 is a membrane-bound ligand critical for osteoclast-osteoblast communication. Upon binding to its receptor EphA2 on osteoblasts, EphrinA2 promotes osteoblast differentiation and bone formation. Dysregulation of the miR-214/miR-206–EphrinA2 axis impairs osteoblast function, disrupts bone remodeling, and can adversely affect the rate and stability of OTM.

In conclusion, elucidating the regulatory functions of miR-214 and miR-206 and their modulation of EphrinA2 provides valuable insights into bone remodeling dynamics during OTM. Targeted manipulation of this pathway holds promise for developing novel molecular therapies that aim to enhance the efficacy, speed, and long-term stability of orthodontic treatments, while also addressing broader skeletal pathologies associated with disrupted bone remodeling.

Keywords: miR-214, miR-206, EphrinA2, osteoblast, orthodontic tooth movement, bone remodeling, microRNA.

Cite this article: Salehivaziri H, et al. Regulation of Osteoblast Differentiation by miR-214 and miR-206 Through EphrinA2 Signaling: Emerging Therapeutic Insights in Orthodontic Tooth Movement. International Journal of Molecular and Cellular Medicine. 2025; 14 (4):0 - 0. DOI: 0

Introduction

Overview of bone remodeling in orthodontic tooth movement

Orthodontic tooth movement (OTM) is a highly regulated biological process that depends on the remodeling of alveolar bone in response to mechanical forces applied to teeth. This remodeling is mediated by a complex interaction of biomechanical stimuli, inflammatory mediators, cellular responses, and gene regulatory networks. When orthodontic force is applied to a tooth, it creates zones of tension and pressure in the periodontal ligament (PDL), which in turn trigger site-specific bone formation and resorption. On the pressure side, bone resorption occurs via the activation of osteoclasts, while on the tension side, osteoblasts promote new bone deposition.

The balance and coordination of these opposing cellular processes are essential for the controlled movement of teeth without damaging the surrounding tissues (1, 2). A key feature of bone remodeling during OTM is the rapid mechanotransduction that converts physical force into biochemical signals. Cells in the PDL and alveolar bone, such as osteoblasts, osteoclasts, fibroblasts, and osteocytes, detect these changes and release a cascade of signaling molecules including cytokines, chemokines, and growth factors that regulate cellular behavior. Well-characterized pathways involved in this process include the RANKL/RANK/OPG axis, which governs osteoclastogenesis, and the Wnt/β-catenin pathway, which regulates osteoblast differentiation and activity (3).More recently, post-transcriptional regulators such as microRNAs (miRNAs) have emerged as essential modulators of bone remodeling (4).

These small, non-coding RNAs can inhibit or enhance the translation of key osteogenic and osteoclastic genes. For instance, miR-214 and miR-206 have been identified as negative regulators of osteoblast differentiation through their targeting of EphrinA2 and other osteogenic transcription factors. These discoveries have opened new avenues for selectively modulating bone remodeling at the molecular level, presenting potential therapeutic strategies to improve the efficiency and safety of orthodontic treatments (5) (Figure 1).

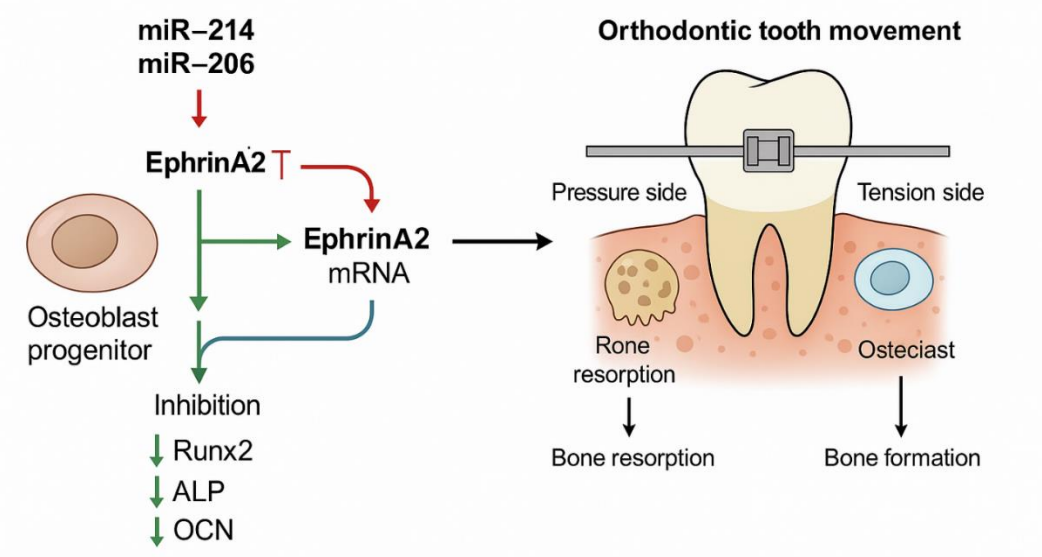

Figure1. Schematic represnation of miR-24 and miR-206 function by EphrinA2 pathway on OTM; Runx2 : Runt-related transcription factor 2; ALP: Alkaline phosphatase; OCN: Osteocalcin.

Orthodontic tooth movement (OTM) is a complex biological and clinical process that faces several challenges in practice. One of the significant issues is biological variability, as patients differ in bone density, periodontal ligament responsiveness, and genetic background, making the rate of movement unpredictable. Age is another factor, as younger patients often respond faster to orthodontic forces compared with adults. Applying the correct magnitude and direction of force is also challenging; too much force causes hyalinization and delays movement, while too little force produces minimal results (6).

Understanding the molecular and cellular mechanisms underlying OTM is not only critical for

clinical orthodontics but also offers insights into fundamental bone biology and the development of skeletal therapeutics. In this review, we provide an up-to-date synthesis of the regulatory systems governing bone remodeling during orthodontic tooth movement, with a particular focus on the emerging role of miRNAs and their potential application in targeted modulation of the remodeling process.

Role of osteoblasts and osteoclasts in alveolar bone dynamics

The alveolar bone undergoes continuous remodeling throughout life and plays a crucial role in supporting the dentition. During orthodontic tooth movement (OTM), this dynamic process is intensified in response to biomechanical stimuli. The two central cellular mediators of this remodeling are osteoblasts, responsible for bone formation, and osteoclasts, responsible for bone resorption. The coordinated activity of these cells ensures the controlled and directional movement of teeth through the alveolar socket without compromising the structural integrity of surrounding tissues (7).

Osteoclasts are large, multinucleated cells derived from the monocyte/macrophage lineage. They are recruited to sites of compressive stress during OTM, where their activity leads to the resorption of alveolar bone. Derived from monocyte/macrophage lineage, responsible for bone resorption and activated by RANKL and M-CSF. The differentiation and activation of osteoclasts are primarily regulated by the RANKL (Receptor Activator of Nuclear Factor κB Ligand) and M-CSF (Macrophage Colony-Stimulating Factor) signaling pathways (8). Osteoblast-lineage cells and periodontal ligament (PDL) fibroblasts secrete RANKL, which binds to RANK receptors on osteoclast precursors, driving their maturation. Osteoclasts then attach to mineralized bone surfaces, form a sealed resorption lacuna, and secrete acid and proteolytic enzymes such as cathepsin K to degrade the bone matrix (9, 10).

Osteoblasts originate from mesenchymal stem cells and are recruited to the tension side of the tooth during OTM. Mesenchymal-derived bone-forming cells. Differentiation is regulated by Runx2, BMPs, and Wnt/β-catenin signaling. Here, they synthesize osteoid—a collagen-rich, unmineralized bone matrix—which subsequently undergoes mineralization to form new bone (11).

Osteoblast differentiation is regulated by signaling molecules such as bone morphogenetic proteins (BMPs) and the Wnt/β-catenin pathway, with key transcription factors including Runx2 and osterix guiding their lineage commitment (3). Mature osteoblasts also produce osteoprotegerin (OPG), a decoy receptor for RANKL, thereby modulating osteoclast activity and maintaining a balanced remodeling environment (11, 12). Bone remodeling during OTM is a tightly coupled process. Osteoclast-mediated resorption releases growth factors embedded in the bone matrix, such as TGF-β and IGF-1, which promote osteoblast recruitment and activation. This interplay ensures a continuous cycle of bone turnover tailored to the directional forces applied during orthodontic treatment (13).

Importance of gene regulation and microRNAs in bone biology

Bone biology is governed by a tightly regulated network of genes that orchestrate the proliferation, differentiation, and function of osteoblasts and osteoclasts. Gene regulation in bone remodeling is a multi-layered process involving transcriptional, epigenetic, and post-transcriptional mechanisms. Among these, microRNAs (miRNAs) have emerged as critical regulators of skeletal development and bone homeostasis, offering novel insights into the molecular basis of bone remodeling during processes such as fracture healing, osteoporosis, and orthodontic tooth movement (OTM) (14).

Key transcription factors such as Runx2, Osterix (Sp7), NFATc1, and PU.1 regulate lineage commitment and activity of bone-forming and bone-resorbing cells. These transcription factors act downstream of signaling pathways, including Wnt/β-catenin, BMP/TGF-β, Notch, and RANK/RANKL/OPG. Their precise temporal and spatial expression ensures proper bone formation and resorption (15). Disruption of this gene regulatory balance can lead to skeletal disorders, such as osteopetrosis, osteoporosis, and abnormal bone turnover during orthodontic therapy. Thus, understanding the regulators that control gene expression is crucial for manipulating bone remodeling therapeutically (16) Summary of bone-regulating miRNAs mentioned in Table 1.

These miRNAs are often differentially expressed in response to mechanical loading, inflammation, or

hormonal changes, making them essential mediators of adaptive bone remodeling. Targeting miRNAs using mimics or antagomirs (inhibitors) opens new avenues for controlling bone remodeling in diseases like osteoporosis or during orthodontic treatments. For instance, inhibition of miR-214 has shown potential in enhancing osteoblast activity and promoting bone formation in experimental models (17).

Table 1. Examples of Bone-Regulating miRNAs

miRNA	Primary Targets	Cell Type	Effect on Bone
miR-214	ATF4, β-catenin, EphrinA2	Osteoblast	↓ Osteoblast activity promotes bone loss(17–20)
miR-206	Runx2, EphrinA2	Osteoblast	↓ Osteogenic differentiation(17–20)
miR-29b	HDAC4, TGF-β3 inhibitors	Osteoblast	↑ Osteoblast differentiation(17–20)
miR-21	PDCD4, PTEN	Osteoclasts	↑ Osteoclastogenesis(17–20)

ATF4: Activating transcription factor 4; Runx2: Runt-related transcription factor 2; HDAC4: Histone Deacetylase 4; TGF-β3 :Transforming Growth Factor beta 3; PDCD4: Programmed cell death protein 4; PTEN:Phosphatase and tensin homolog.

miR-214: miR-206, and EphrinA2

Emerging evidence highlights the critical role of microRNAs (miRNAs) in modulating bone remodeling during orthodontic tooth movement (OTM), particularly through the regulation of osteogenic differentiation and osteoclast function. Among these, miR-214 and miR-206 have attracted significant attention for their inhibitory effects on osteoblast activity and their interaction with the EphrinA2-Eph receptor signaling axis. This review focuses on the molecular mechanisms through which miR-214 and miR-206 influence alveolar bone remodeling, with a special emphasis on their shared target, EphrinA2, and its implications for therapeutic modulation of orthodontic treatment (17, 18).

miR-214: A Suppressor of Osteoblast Function

miR-214 is one of the most consistently reported miRNAs negatively regulating bone formation. Expressed primarily in osteoclasts and to some extent in osteoblasts, miR-214 exerts a paracrine inhibitory effect on osteoblast activity. Its known targets include ATF4, β-catenin, and EphrinA2, all critical regulators of osteogenesis.

In particular, miR-214-mediated suppression of EphrinA2 disrupts bidirectional signaling with Eph receptors (EphA2) on osteoblasts, impairing their differentiation and mineralization capacity. Elevated levels of miR-214 have been associated with bone loss in conditions such as osteoporosis and are increasingly implicated in force-induced bone remodeling during OTM (19-21).

miR-206: A Regulator of Myogenesis with Osteogenic Crosstalk

Primarily known for its role in muscle differentiation, miR-206 also modulates bone biology by targeting osteogenic transcription factors such as Runx2 and EphrinA2. Its expression is induced in response to mechanical stress and inflammation, both of which are present during OTM. Similar to miR-214, miR-206 reduces osteoblast proliferation and differentiation. By downregulating EphrinA2, miR-206 indirectly impairs the EphrinA2-EphA2 signaling pathway, limiting osteoblast activation in areas subjected to tensile forces (19, 22).

EphrinA2: A Key Mediator of Osteoblast–Osteoclast Crosstalk

EphrinA2, a membrane-bound ligand of the Eph receptor family, plays a crucial role in the sophisticated crosstalk between osteoclasts and osteoblasts. It is expressed on the surface of osteoclasts and binds to EphA2 receptors on osteoblasts, initiating reverse signaling that promotes osteoblast differentiation and bone formation. Disruption of this axis—such as through overexpression of miR-214 or miR-206—attenuates bone formation and leads to an imbalance in bone remodeling, which can affect the efficiency and stability of orthodontic tooth movement (23, 24).

Understanding the regulatory axis of miR-214/miR-206–EphrinA2 provides a molecular framework for selectively modulating bone remodeling during orthodontic treatment. Targeted inhibition of these miRNAs could enhance osteoblastic activity,

accelerate bone deposition on the tension side, and improve orthodontic outcomes. This has potential implications not only for optimizing treatment timelines but also for managing conditions with compromised bone remodeling, such as in adult or osteopenic patients undergoing orthodontic therapy (20, 22).

EphrinA2 significantly influences osteoblast differentiation through its role as a membrane-bound ligand of the Eph receptor family, mediating communication between osteoblasts and osteoclasts. This influence is primarily exerted via forward and reverse signaling mechanisms, both of which are crucial for bone formation and mineralization (23).

Forward Signaling and Osteogenesis

Mechanism: When EphrinA2 binds to EphA2 receptors located on osteoblasts, it initiates intracellular cascades (25). Key Transcription Factors: This binding triggers the activation of crucial transcription factors such as Runx2 and Osterix (Sp7), which are essential drivers of osteoblast differentiation and maturation.

Wnt/β-catenin Pathway Activation: EphrinA2 stimulation also activates the Wnt/β-catenin signaling pathway, further enhancing bone formation and mineralization (26).

Reverse Signaling and Osteoblast Behavior

Modulation of Cell Adhesion and Migration: Reverse signaling, initiated by EphrinA2, affects osteoblast behavior by modulating integrin-mediated cell adhesion and migration, processes vital for bone matrix remodeling and repair (27).

Osteocalcin Upregulation: This signaling also upregulates osteocalcin, a protein that is critical for bone mineral quality (28). EphrinA2's bidirectional signals finely tune osteoblast function, contributing to the dynamic process of bone homeostasis. Its proper function ensures the coupling of bone formation and resorption, which is essential for maintaining skeletal integrity.

Disruptions in this pathway, such as those caused by overexpression of miR-214 or miR-206, can attenuate bone formation and lead to an imbalance in bone remodeling. Therefore, restoring EphrinA2 expression is considered a potential therapeutic strategy to enhance osteoblast-driven bone formation (27).

MicroRNA-Mediated Regulation of Bone Remodeling

MicroRNAs (miRNAs) are small (~22 nucleotides), non-coding RNAs that function as potent post-transcriptional regulators of gene expression. By binding to complementary sequences in the 3′ untranslated region (3′ UTR) of target messenger RNAs (mRNAs), miRNAs lead to mRNA degradation or translational repression. In the context of bone biology, miRNAs have emerged as critical modulators of both osteoblast-mediated bone formation and osteoclast-mediated bone resorption.

These regulatory functions position miRNAs as central players in skeletal development, homeostasis, and the bone remodeling processes involved in orthodontic tooth movement (OTM) (29) (Table 1). Upon mechanical stimulation, intracellular signaling pathways—including MAPK/ERK, NF-κB, Wnt/β-catenin, and TGF-β/Smad—are activated, which regulate transcription factors that control the expression of specific microRNAs (miRNAs). Mechanical stress can modulate miRNA expression at multiple levels, including transcription, epigenetic regulation, and post-transcriptional processing via Drosha and Dicer. For example, compression forces upregulate miR-21 in PDL cells, promoting osteoclastogenesis, while tension forces regulate miR-34a and miR-29b, which influence osteoblast differentiation and extracellular matrix deposition.

Additionally, stress-induced miRNAs such as miR-146a participate in fine-tuning inflammatory responses during OTM. Collectively, mechanically regulated miRNAs orchestrate bone remodeling, PDL adaptation, and inflammatory signaling, thereby controlling the rate and extent of tooth movement (30, 31). So, mechanical forces in OTM are sensed by PDL and bone cells, triggering signaling cascades (MAPK, NF-κB, Wnt, TGF-β) which in turn modulate transcription, processing, and stability of specific miRNAs. These miRNAs then regulate osteogenesis, osteoclastogenesis, ECM remodeling, and inflammatory responses, ultimately controlling tooth movement (32).

Mechanisms of Action in Bone Cells

In osteoblasts, miRNAs regulate key transcription factors and signaling pathways involved in differentiation, such as Runx2, Osterix (Sp7), and components of the Wnt/β-catenin and BMP/TGF-β

signaling axes (33). For example: miR-29b promotes osteoblastogenesis by repressing inhibitors of osteoblast differentiation. miR-133 and miR-206 negatively regulate Runx2, leading to inhibition of osteoblastic maturation (34). In osteoclasts, miRNAs such as miR-21, miR-223, and miR-148a modulate genes involved in differentiation and bone-resorbing activity through pathways like RANKL/ RANK/NFATc1 (35).

MicroRNAs in Orthodontic Bone Remodeling

miR-214 impairs osteoblast function by targeting ATF4, β-catenin, and EphrinA2, reducing bone formation on the tension side. miR-206 inhibits osteogenic differentiation by targeting Runx2 and EphrinA2, especially under mechanical stress. miR-21 facilitates osteoclastogenesis, promoting bone resorption on the pressure side. These miRNAs integrate mechanical cues with molecular signaling, acting as "fine-tuners" of the remodeling balance (17, 20).

Therapeutic Implications

Given their specificity and regulatory potency, miRNAs are being explored as therapeutic targets in skeletal disorders and orthodontics. Synthetic miRNA mimics or inhibitors (antagomirs) offer potential strategies to enhance or suppress remodeling activities at specific sites selectively. For instance, antagonizing miR-214 in vivo has been shown to improve bone formation, suggesting a viable approach to accelerate post-orthodontic stabilization or treat bone loss (19).

Biological Roles of miR-214 and miR-206 in Bone Formation

MicroRNAs (miRNAs) are small, non-coding RNA molecules that fine-tune gene expression post-transcriptionally, playing pivotal roles in bone development, remodeling, and disease. Among these, miR-214 and miR-206 have emerged as critical regulators of bone metabolism, influencing osteoblast differentiation, osteoclast activity, and the intricate crosstalk between muscle and bone.

miR-214: A Dual Regulator of Bone Formation and Resorption

Suppression of Osteoblast Differentiation and Bone Formation : miR-214 acts as a potent inhibitor of osteogenesis by targeting multiple osteogenic pathways: ATF4 (Activating Transcription Factor 4): A master regulator of osteoblast function and bone matrix synthesis; miR-214 downregulates ATF4, impairing osteoblast maturation (17). Osterix (Sp7): A transcription factor essential for osteoblast differentiation; miR-214 suppresses Sp7, reducing bone formation (21).

Wnt/β-catenin signaling: By inhibiting β-catenin, miR-214 disrupts a key anabolic pathway in bone development (36). Elevated miR-214 levels are observed in osteoporotic patients, correlating with reduced bone mineral density (BMD). Genetic deletion or pharmacological inhibition of miR-214 enhances bone formation in murine models, suggesting therapeutic potential (37, 38).

Promotion of Osteoclast Activity and Bone Resorption

Beyond suppressing osteoblasts, miR-214 enhances osteoclastogenesis via:

PTEN inhibition: By downregulating PTEN (a negative regulator of osteoclastogenesis), miR-214 activates the PI3K/Akt pathway, promoting osteoclast survival and bone resorption. NF-κB signaling: miR-214 may also amplify pro-osteoclastogenic signals through NF-κB activation. The dual role of miR-214—inhibiting bone formation while stimulating resorption—makes it a major contributor to bone loss in osteoporosis and inflammatory bone diseases (20).

miR-206: A Key Player in Osteoblast Inhibition and Muscle-Bone Crosstalk

Inhibition of Osteoblast Differentiation

miR-206 suppresses bone formation by targeting (39): Connexin 43 (Cx43): A gap junction protein critical for osteoblast communication and differentiation; miR-206 downregulates Cx43, impairing osteoblast function. Runx2: A master transcription factor for osteogenesis; miR-206 reduces Runx2 expression, blocking osteoblast maturation. Disuse osteoporosis: miR-206 is upregulated in mechanical unloading (e.g., bed rest, microgravity), contributing to bone loss by suppressing osteoblast activity.

Role in Muscle-Bone Interaction

Emerging evidence suggests miR-206 mediates sarcopenia-related bone loss (40): Exosomal transfer: Skeletal muscle secretes miR-206 via exosomes, which

can be taken up by osteoblasts, inhibiting their function. Myokine-like effects: miR-206 may act as a signaling molecule in muscle-bone crosstalk, linking muscle atrophy to bone deterioration. Therapeutic potential: Anti-miR-206 strategies could mitigate disuse-induced bone loss and sarcopenia-related osteoporosis.

Targeting miR-214 for Bone Anabolism

AntagomiR-214: Preclinical studies show that miR-214 inhibition enhances bone formation and reduces resorption. Delivery systems: Nanoparticle-based miR-214 inhibitors are being explored for osteoporosis treatment. miR-206 blockade: Could promote osteogenesis in immobilized patients or astronauts. Exercise-induced downregulation: Physical activity reduces miR-206, suggesting exercise as a natural modulator (19, 40, 41).

Challenges and Future Research

Tissue-specific delivery: Avoiding off-target effects remains a hurdle. Dual targeting: Simultaneously inhibiting miR-214 and miR-206 may offer synergistic benefits. miR-214 and miR-206 are crucial regulators of bone homeostasis, with miR-214 acting as a dual inhibitor of osteogenesis and promoter of osteoclast activity, while miR-206 suppresses osteoblast function and mediates muscle-bone crosstalk. Their dysregulation contributes to osteoporosis, disuse-induced bone loss, and sarcopenia-related bone deterioration. Targeting these miRNAs holds significant therapeutic promise, though further research is needed to optimize delivery and minimize side effects. Understanding their mechanisms opens new avenues for treating bone metabolic disorders (42).

So, the role of microRNAs (miRNAs) in orthodontic tooth movement (OTM) presents conflicting evidence and unanswered questions, particularly regarding their mechanosensitivity and regulatory functions. While studies indicate that miRNAs, such as miR-21, are crucial in mediating responses to mechanical forces during OTM, the exact mechanisms and interactions with other signaling pathways remain unclear. miR-21 has been shown to respond to orthodontic forces, influencing osteogenic differentiation and osteoclastogenesis in periodontal tissues. However, the extent to which other miRNAs participate in this mechanotransduction process is not fully understood, leaving a gap in knowledge about their collective roles (43).

The interplay between miRNAs and inflammatory responses during OTM is complex. For instance, miR-21 mediates accelerated OTM under inflammatory conditions, yet the broader implications of inflammation on miRNA activity are not well defined. The potential for dysregulated inflammation leading to adverse outcomes, such as root resorption, raises questions about the balance between miRNA activity and inflammatory responses. Despite the advancements in understanding miRNAs in OTM, the complexity of their interactions with various biological processes and the need for more comprehensive studies highlight the ongoing challenges in this field (32).

EphrinA2 Signaling Pathway in Bone Homeostasis

Bone homeostasis is a dynamic process regulated by the balanced activities of osteoblasts and osteoclasts, responsible for bone formation and resorption, respectively. The Eph-ephrin family of receptor tyrosine kinases, particularly EphrinA2, has emerged as a key mediator of cell-cell communication in this remodeling process. EphrinA2 signaling orchestrates bidirectional interactions between osteoblasts and osteoclasts, thereby maintaining skeletal integrity. Dysregulation of this pathway has been linked to bone diseases such as osteoporosis, metastatic bone lesions, and delayed fracture healing. This review discusses the molecular mechanisms of EphrinA2 in bone metabolism and evaluates its potential as a therapeutic target (27).

EphrinA2 Signaling in Osteoblasts

EphrinA2 exerts its effects through forward and reverse signaling mechanisms. Forward signaling is initiated when ephrinA2 binds to EphA2 receptors on osteoblasts, triggering intracellular cascades that promote osteogenesis. This includes the activation of key transcription factors Runx2 and Osterix (Sp7), which drive osteoblast differentiation and maturation. Additionally, EphrinA2 stimulation activates the Wnt/β-catenin signaling pathway, further enhancing bone formation and mineralization (27). Conversely, reverse signaling by ephrinA2 affects osteoblast behavior by modulating integrin-mediated cell adhesion and migration, critical for bone matrix remodeling and repair. Reverse signaling also upregulates osteocalcin, a protein essential for bone

mineral quality. Together, these bidirectional signals finely tune osteoblast function (44).

Regulation of Osteoclast Activity by EphrinA2

In osteoclasts, EphrinA2-EphA2 interactions inhibit osteoclastogenesis, helping to prevent excessive bone resorption. This inhibition occurs through the downregulation of NFATc1 and c-Fos, transcription factors essential for osteoclast differentiation. EphrinA2 signaling also blocks RANKL-induced activation of MAPK and NF-κB pathways, further suppressing osteoclast formation and activity. By coordinating these effects, EphrinA2 ensures the coupling of bone formation and resorption, maintaining skeletal homeostasis (44).

Implications in Bone Pathologies

Osteoporosis: Reduced expression of ephrinA2 in osteoporotic bone disrupts this balance, leading to impaired osteoblast differentiation and unchecked osteoclast activity, resulting in bone loss and increased fracture risk. Bone Metastasis: Certain tumors exploit ephrinA2 signaling to promote osteolytic lesions. Breast and prostate cancers upregulate ephrinA2 to facilitate bone colonization and destruction. Targeting ephrinA2 with neutralizing antibodies or siRNA shows promise in reducing metastatic bone damage (45, 46). Fracture Healing: EphrinA2 promotes fracture repair by recruiting osteoprogenitor cells to the callus and enhancing new bone formation. Deficiency in ephrinA2 delays healing and increases the risk of non-union fractures, indicating potential for ephrinA2-based therapies in bone regeneration (45, 46).

Therapeutic Potential

Efforts to modulate ephrinA2 signaling therapeutically are underway. Recombinant ephrinA2-Fc fusion proteins enhance osteogenesis in preclinical models, offering a potential anabolic treatment for osteoporosis. Small-molecule EphA2 agonists are also in development, aimed at stimulating bone formation. Conversely, antagonists such as EphA2-neutralizing antibodies and siRNA-based ephrinA2 silencing demonstrate efficacy in reducing tumor-induced osteolysis, presenting a novel approach for treating bone metastases (45).

EphrinA2 is a crucial regulator of bone remodeling, balancing osteoblast and osteoclast functions through complex bidirectional signaling. Its dysregulation contributes to major bone disorders, highlighting its significance as a therapeutic target. Future research should focus on developing tissue-specific delivery systems for ephrinA2 modulators to maximize therapeutic benefits while minimizing off-target effects (45-47).

EphA2 is overexpressed in various bone sarcomas, including osteosarcoma, Ewing sarcoma, and chondrosarcoma. Higher EphA2 expression correlates with advanced disease stages and poor prognosis. Targeting EphA2 has been proposed as a therapeutic strategy to inhibit tumor growth and metastasis in these malignancies. While direct evidence of polymorphisms or mutations in EphrinA2/EphA2 affecting bone metabolism is limited, their expression and signaling play significant roles in bone remodeling and pathology. Further research is needed to identify specific genetic variations and their implications in bone health (45-47).

Mechanistic Interplay Between miR-214/miR-206 and EphrinA2

The Ephrin–Eph signaling pathway plays a central role in regulating bone remodeling by mediating communication between osteoclasts and osteoblasts. In this context, EphrinA2, a membrane-bound ligand expressed on osteoclasts, and its receptor EphA2, present on osteoblasts, form a bidirectional signaling system that promotes osteoblast differentiation and suppresses excessive bone resorption.

Disruption of this signaling axis by specific microRNAs, particularly miR-214 and miR-206, has been shown to impair osteogenic activity and alter bone homeostasis (44, 48, 49). Based on Figure 2, the effects of miR-214 and miR-206 on osteoblasts and osteoclasts are explained (Figure 2).

During orthodontic tooth movement (OTM), miR-214 and miR-206 play critical roles in regulating bone remodeling by modulating osteoclastogenesis and osteoblast differentiation. Inhibition of these miRNAs could potentially alter the balance between bone resorption and formation.

However, the regulatory network governing bone remodeling is highly redundant, and other miRNAs and signaling pathways may compensate for their inhibition. For instance, miR-21 promotes osteoclast differentiation by targeting PDCD4 and enhancing RANKL signaling, which could partially offset reduced osteoclast activity resulting from miR-214

inhibition. At the signaling level, pathways such as RANK/RANKL/OPG, Wnt/β-catenin, MAPK/ERK, PI3K/AKT, and TGF-β/BMP are activated during OTM and can maintain osteoclast and osteoblast activity despite miRNA inhibition. These overlapping regulatory mechanisms ensure that bone remodeling proceeds under mechanical stress, although the rates of resorption and formation may be altered. Understanding these compensatory networks is crucial for developing targeted therapeutic strategies to modulate tooth movement effectively (42, 44, 48).

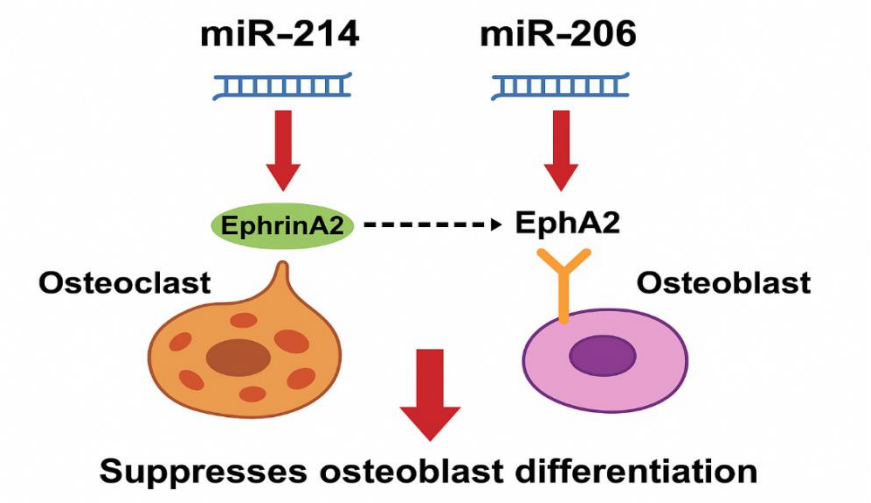

Figure 2. The effect of miR-214 and miR-206 on the osteoblasts and osteoclasts

miR-214: Suppressing EphrinA2 and Osteoblast Differentiation

miR-214 is predominantly expressed in osteoclasts and is released in extracellular vesicles to exert paracrine effects on neighboring osteoblasts. One of its key molecular targets is EphrinA2, which plays a pivotal role in osteoblast activation through reverse signaling. When miR-214 binds to the 3′ untranslated region (3′ UTR) of EphrinA2 mRNA, it suppresses its translation, thereby impairing the EphrinA2–EphA2 interaction.

This leads to reduced osteoblast differentiation via the downregulation of downstream transcription factors, such as Runx2 and ATF4, decreased bone formation, especially on the tension side during orthodontic tooth movement (OTM), and a potential enhancement of osteoclast-mediated bone resorption due to uncoupled remodeling (41, 42, 44).

miR-206: Dual Regulator of Muscle and Bone

Although miR-206 is traditionally associated with myogenesis, it has emerged as a negative regulator of osteoblastogenesis. Like miR-214, miR-206 also targets EphrinA2, reducing its availability for interaction with EphA2 receptors on osteoblasts. Additionally, miR-206 represses Runx2, a master regulator of osteoblast differentiation, leading to an additive inhibitory effect on bone formation.

This dual repression amplifies the inhibitory signal on osteoblast maturation, attenuates the anabolic response required for effective bone deposition during orthodontic force application, and potentially prolongs treatment duration or compromises post-treatment stability (18, 50).

Combined Effect on Bone Remodeling

Therapeutic Perspectives

Targeted inhibition of miR-214 and/or miR-206 through antagomirs, locked nucleic acid inhibitors, or miRNA sponges has shown promise in preclinical models. Restoring EphrinA2 expression through these approaches could enhance osteoblast-driven bone formation, providing a novel molecular tool to improve bone remodeling efficiency in orthodontics and other skeletal disorders.

The miR-206 and miR-214 targets and pathways involved in osteoblast differentiation are listed in Table 2. As demonstrated in Table 3, the roles of miR-214 and miR-206 in bone remodeling via EphrinA2 are explained (Table 3)(51).

Implications for Orthodontic Tooth Movement

The prolonged modulation of microRNAs (miRNAs) in orthodontic interventions presents

Extended inhibition or overexpression could inadvertently compromise unrelated signaling cascades, thereby affecting bone homeostasis, angiogenesis, or even systemic organ functionality. For instance, excessive repression of miR-214 aimed at diminishing osteoclast activity might disrupt normal bone remodeling processes in other regions, resulting in localized osteopetrosis or delayed bone turnover. Another significant issue is immune activation (51).

The introduction of miRNA mimics or antagonists, particularly through nanoparticles or viral vectors, may elicit recognition as foreign entities by the immune system, potentially inciting inflammation or hypersensitivity responses. Persistent local immune activation in the periodontal ligament could exacerbate tissue injury or impede orthodontic tooth movement. Furthermore, cellular toxicity could emerge if delivery systems accumulate or degrade at a sluggish pace, possibly influencing fibroblasts, osteoblasts, or osteoclasts (52).

Unforeseen ramifications on angiogenesis or nerve signaling within the periodontal ligament may also jeopardize dental vitality or periodontal integrity. Lastly, enduring epigenetic modifications represent a theoretical hazard, as continuous miRNA modulation might instigate compensatory alterations in gene expression that extend beyond the therapeutic timeframe. To mitigate these risks, methodologies should emphasize localized, regulated, and transient delivery of miRNAs, coupled with stringent preclinical safety evaluations and the implementation of biocompatible, degradable carriers that minimize systemic exposure and off-target consequences (52).

Table 2. miR-206 and miR-214 targets and pathways on osteoblast differentiation

miRNA	Primary Target(s)	Pathway Affected	Effect on Osteoblast Differentiation	Downstream Markers Affected
miR-214	EphrinA2 mRNA	Eph/Ephrin signaling	↓Inhibits differentiation	↓ Runx2, ↓ ALP, ↓ OCN
	ATF4, β-catenin	Wnt/β-catenin, ATF4 axis	↓Suppresses osteoblast maturation	↓ Osteopontin, ↓ OCN
miR-206	EphrinA2 mRNA	EphrinA2/ERK signaling	↓ Inhibits early differentiation	↓ Runx2, ↓ ALP
	Connexin43 (GJA1)	Gap junction communication	↓ Reduces osteoblast function	↓ ALP

ATF4: Activating transcription factor 4; Runx2: Runt-related transcription factor 2; ALP: Alkaline phosphatase; OCN: Osteocalcin.

Table 3. Comparative Roles of miR-214 and miR-206 in Bone Remodeling via EphrinA2

Feature/Aspect	miR-214	miR-206
Primary Expression	Osteoclasts	Muscle cells, osteoblasts
Experimental Evidence	In Vitro Differentiation of Osteoclasts and Osteoblasts	In Vitro
Key Target(s)	EphrinA2, ATF4, β-catenin	EphrinA2, Runx2
Mechanism of Action	Inhibits osteoblast differentiation via suppression of EphrinA2-mediated signaling	Inhibits osteoblastogenesis by targeting EphrinA2 and Runx2

Feature/Aspect	miR-214	miR-206
Effect on EphrinA2-EphA2 Axis	Downregulates EphrinA2 in osteoclasts, disrupting reverse signaling to osteoblasts.	Downregulates EphrinA2 in osteoblasts, limiting EphA2 interaction.
Net Effect on Bone Remodeling	Decreases bone formation; favors resorption	Inhibits osteogenic differentiation
Relevance to OTM	Impairs tension-side bone formation	Attenuates bone deposition during OTM
Therapeutic Potential	Antagomir enhances osteoblast activity	Antagomir may improve bone anabolic response
Delivery Challenge	Targeting vesicle-based intercellular miRNA transfer	Specific inhibition in bone tissue is required

ATF4: Activating transcription factor 4; OTM: Orthodontic Tooth Movement; Runx2: Runt-related transcription factor 2.

Alveolar Bone Dynamics Under Mechanical Force

Orthodontic tooth movement (OTM) is a biologically mediated process driven by the application of controlled mechanical forces to teeth. These forces generate stress in the periodontal ligament (PDL) and surrounding alveolar bone, leading to site-specific bone remodeling (53). The pressure side of the PDL induces osteoclast recruitment and bone resorption, while the tension side promotes osteoblast differentiation and bone formation. This coordinated remodeling maintains alveolar bone integrity and enables controlled tooth displacement.

Mechanotransduction—the conversion of mechanical signals into biochemical responses—activates a cascade of signaling pathways, including RANKL/OPG, Wnt/β-catenin, and MAPK, influencing gene expression and cellular behavior. Emerging evidence suggests that mechanical loading also modulates non-coding RNAs, particularly microRNAs (miRNAs), which play a crucial role as post-transcriptional regulators during OTM (32).

Modulating Bone Remodeling Using miRNAs: A Future Strategy

miRNAs are capable of fine-tuning gene expression in response to orthodontic force. Their dynamic expression profiles during OTM suggest that they could be leveraged to modulate bone remodeling therapeutically. For instance, miR-214 and miR-206 downregulate osteoblast differentiation by targeting EphrinA2, Runx2, and ATF4—key players in bone formation.

Inhibiting these miRNAs could enhance osteogenesis on the tension side, accelerating tooth movement and reducing treatment time. Conversely, promoting specific miRNAs that encourage osteoclast apoptosis or suppress excessive bone resorption could enhance post-treatment stability. miRNA-based modulation holds promise for personalized orthodontic interventions, especially in patients with delayed tooth movement, poor bone density, or metabolic bone disorders (54).

Potential Clinical Applications: miRNA Mimics, Antagomirs, and Targeted Delivery

Therapeutic manipulation of miRNAs can be achieved using miRNA mimics (to restore downregulated beneficial miRNAs) or antagomirs (chemically modified antisense oligonucleotides that inhibit specific miRNAs). For instance, antagomirs targeting miR-214 have shown efficacy in restoring osteoblast activity and bone formation in preclinical models. Effective delivery systems—such as lipid nanoparticles, hydrogels, or exosome-based carriers—are crucial to ensure targeted release to periodontal tissues. Localized delivery minimizes systemic side effects and enhances therapeutic concentration at the site of force application. The integration of miRNA therapy with current orthodontic appliances, such as brackets or aligners coated with miRNA-delivering materials, represents a futuristic yet plausible approach (54, 55). Summary of miRNA-based strategies and their clinical implications in Orthodontic Tooth Movement is mentioned in Table 4.

Table 4. Summary of miRNA-Based Strategies and Their Clinical Implications in Orthodontic Tooth Movement

Aspect	Description
Target miRNAs	miR-214, miR-206, miR-29, miR-21, miR-34a, etc.
Mechanisms of Action	Regulation of osteoblast/osteoclast differentiation; modulation of EphrinA2, Runx2, RANKL
Therapeutic Tools	miRNA mimics (agonists), antagomirs (antagonists), locked nucleic acid (LNA) inhibitors
Delivery Systems	Nanoparticles, hydrogels, exosome-based carriers, scaffold-based, or aligner coatings
Potential Clinical Benefits	Accelerated tooth movement, enhanced bone regeneration, improved post-treatment stability.
Local vs Systemic Delivery	Preference for local delivery to minimize systemic side effects
Challenges	Off-target gene effects, miRNA stability, delivery efficiency, immunogenicity, and regulatory approval
Clinical Translation Status	Preclinical studies are promising; human trials and orthodontic-specific applications are pending.

Runx2: Runt-related transcription factor 2; RANKL: Receptor Activator of Nuclear factor κB Ligand.

Challenges in Translation to Human Therapies

Despite promising experimental data, several barriers remain before miRNA-based therapies can be implemented clinically in orthodontics (56): Stability and degradation: miRNAs are prone to rapid degradation in biological fluids, necessitating protective delivery vectors.

Tissue specificity: Achieving targeted delivery to alveolar bone or periodontal ligament without affecting other tissues remains a technical hurdle. Off-target effects: miRNAs often regulate multiple genes; unintended modulation of unrelated pathways could cause adverse effects. Regulatory and ethical considerations: The translation of nucleic acid-based therapeutics into human use requires rigorous validation, long-term safety studies, and regulatory approvals(56).

microRNA (miRNA)-based methods show great potential for improving orthodontic treatments by precisely controlling bone remodeling at the cellular level. An auspicious approach involves using miRNA-encapsulated orthodontic brackets. In this method, brackets or archwires can be coated with biodegradable layers that contain miRNA mimics or inhibitors, allowing targeted, localized release within the periodontal ligament (PDL).

For example, administering miR-214 inhibitors could be aimed at areas needing reduced osteoclast activity, helping to decrease excessive bone resorption. At the same time, releasing miR-29 mimics could promote osteoblast differentiation and stimulate bone growth in specific regions (17, 54). Another approach involves injecting miRNAs directly into the PDL or alveolar bone near moving teeth. This method offers precise control of timing and location, reducing systemic exposure and off-target effects. Using nanoparticle carriers like liposomes, chitosan, or hydrogel systems can stabilize miRNAs, protect against degradation, and ensure effective delivery to target cells. Furthermore, combining mechanical stimulation with miRNA therapy could enhance results. For instance, applying controlled orthodontic forces alongside miRNA release can optimize bone formation or prevent resorption. Future developments might include innovative biomaterials that respond to mechanical forces or inflammatory signals, enabling on-demand miRNA delivery for highly personalized tooth movement regulation (17, 54).

In conclusion, integrating miRNA-based therapies into orthodontic practice could lead to faster, more precise, and biologically guided tooth movement, while also reducing side effects such as root resorption

or alveolar bone loss. Although miRNA-targeted strategies hold significant promise for modulating OTM, further preclinical research and clinical trials are needed to confirm their safety, feasibility, and effectiveness.

Therapeutic Potential and Future Directions

miRNA-based diagnostics and therapeutics in orthodontics

MicroRNAs (miRNAs) have emerged as promising molecular tools in orthodontics, offering novel avenues for both diagnosis and therapy. Their regulatory roles in gene expression linked to bone remodeling, inflammation, and tissue repair make them valuable biomarkers for monitoring orthodontic tooth movement and associated biological processes (57, 58).

Recent studies have highlighted the potential of miRNAs detectable in easily accessible fluids, such as saliva and gingival crevicular fluid, enabling non-invasive, real-time assessment of orthodontic treatment progress.

For instance, miR-146a-5p, detectable in periodontal tissues, has been identified as a potential biomarker for periodontal conditions due to its role in modulating osteogenesis and inflammation via the BMP6/Smad signaling pathway, offering opportunities for non-invasive monitoring and targeted therapeutic interventions in orthodontics (51, 59). Summary of experimental studies related to miRNAs on osteoblast and osteoclasts manifested in Table 5.

Integration with biomaterials, gene therapy, or nanodelivery systems

The clinical translation of miRNA-based orthodontic therapies may be significantly enhanced by integrating them with advanced biomaterials, gene therapy techniques, and nanodelivery platforms. Biomaterials, such as biodegradable scaffolds and membranes used in guided tissue regeneration, can be functionalized to release miRNAs in a controlled manner, promoting targeted bone and periodontal ligament remodeling. Smart biomaterials responsive to mechanical forces present an exciting prospect for on-demand miRNA delivery, which can be synchronized with the phases of orthodontic treatment (60). Moreover, combining miRNA modulation with gene-editing technologies like CRISPR/Cas9 could address genetic predispositions that influence treatment outcomes. Nanotechnology offers versatile delivery vehicles—liposomes, polymeric nanoparticles, and chitosan-based carriers—that protect miRNAs from enzymatic degradation, enhance cellular uptake, and enable site-specific targeting. Preclinical models have demonstrated improved therapeutic efficacy and reduced systemic side effects using these nanodelivery systems, underscoring their potential in future orthodontic applications (61).

Table 5. Summary of experimental studies related to miRNAs on osteoblasts and osteoclasts

Reference	Study Type	miRNAs Investigated	Key Findings	Methodology	Limitations
Zhang et al., 2021 (50)	Experimental (in vitro & in vivo)	miR-21, miR-29b	Demonstrated miR-21 upregulation promotes osteoclast differentiation, accelerating bone resorption during orthodontic tooth movement; miR-29b enhances osteoblast differentiation, favoring bone formation. This suggests a coordinated miRNA regulation of remodeling processes.	qPCR analysis of miRNA expression; histological staining for bone changes; rat orthodontic tooth movement model	Findings are limited to the rat model; short observation period; human relevance requires further validation.

Reference	Study Type	miRNAs Investigated	Key Findings	Methodology	Limitations
Liu et al., 2022 (51)	Clinical observational study	miR-155, miR-146a	Reported significant elevation of pro-inflammatory miR-155 in gingival crevicular fluid (GCF) correlated with clinical signs of inflammation during active orthodontic treatment. MiR-146a showed anti-inflammatory trends, indicating potential as biomarkers for monitoring tissue response.	Collection of GCF samples from orthodontic patients; RT-qPCR quantification of miRNAs	Small patient cohort; cross-sectional design; lack of functional follow-up.
Cao et al., 2021 (52)	In vitro cellular study	miR-140-5p	Identified miR-140-5p as a negative regulator of osteoclastogenesis, capable of suppressing differentiation and activity of osteoclasts. Suggests therapeutic potential to reduce root resorption, a common adverse effect in orthodontics.	Culture of osteoclast precursor cells; transfection with miRNA mimics and inhibitors; assessment of differentiation markers	Absence of in vivo experiments; translation to clinical application still uncertain.
Singh et al., 2024 (53)	Review article	Multiple miRNAs (e.g., miR-21, miR-29, miR-155)	Comprehensive review summarizing miRNA involvement in bone remodeling, inflammation modulation, and pain regulation during orthodontic tooth movement. Highlights gaps in clinical translation and need for standardized protocols.	Systematic literature review of preclinical and clinical studies	Limited by the quantity and quality of available clinical data, calls for future research.
Puranik et al., 2024 (54)	Animal study (rodent model)	miR-34a, miR-125b	Found miR-34a promotes osteoblast proliferation and bone formation, accelerating tooth movement; miR-125b acts conversely by inhibiting osteoblast differentiation. Demonstrates opposing roles of miRNAs in bone remodeling during orthodontic forces.	Induction of tooth movement in rats; gene expression assays; bone histomorphometry	Species-specific differences may affect extrapolation to humans; requires further validation.

Reference	Study Type	miRNAs Investigated	Key Findings	Methodology	Limitations
Chen et al., 2024 (55)	Pilot clinical trial	miR-27b	Showed that salivary miR-27b levels correlate with the rate of orthodontic tooth movement, suggesting its potential use as a non-invasive biomarker to personalize treatment duration and force application.	Longitudinal collection of saliva from patients; qPCR-based miRNA quantification; clinical measurement of tooth movement	Limited sample size and duration; further large-scale studies needed for validation.

Need for in vivo models and clinical trials

While in vitro studies have provided foundational insights into miRNA functions during orthodontic tooth movement, their translational value is limited without robust in vivo validation. Most current animal models are small rodents whose dental and skeletal anatomy only partially recapitulates human orthodontic biomechanics. The development of larger animal models with closer resemblance to human dentofacial structures is essential to evaluate the long-term efficacy, safety, and optimal dosing of miRNA-based interventions. Furthermore, the progression to clinical trials remains a critical step. Early-phase trials should focus on establishing safety profiles, tolerability, and preliminary therapeutic efficacy in human subjects undergoing orthodontic treatment. Given the novel nature of gene and miRNA therapies, rigorous regulatory oversight and ethical considerations will play a crucial role in shaping clinical development pathways (52).

To evaluate miRNA-based orthodontic therapies in a clinically relevant context, large-animal models that closely mimic human craniofacial anatomy and bone remodeling are essential. Beagle dogs are commonly used because of their similar tooth morphology, periodontal ligament thickness, and bone turnover rates, allowing assessment of localized miRNA delivery via coated brackets or injections and monitoring of tooth movement and root resorption.

Miniature pigs, such as Göttingen pigs, offer comparable craniofacial anatomy and bone density, enabling detailed imaging of alveolar bone changes over time and evaluation of therapy efficacy and safety (62). For the closest approximation to human physiology, non-human primates like macaques can model complex orthodontic biomechanics and immune responses to miRNA delivery, supporting long-term safety studies. While smaller rodent models remain valuable for mechanistic studies, including the effects of specific miRNAs on osteoclast and osteoblast activity. A tiered approach, starting with rodent models for mechanistic insights and advancing to larger animals for translational assessment, can optimize miRNA delivery strategies, dosing, and safety profiles, ultimately bridging the gap toward human clinical trials (63).

Personalized medicine approaches in orthodontics

Orthodontic treatment outcomes vary widely among individuals, influenced by genetic, epigenetic, and environmental factors. The incorporation of miRNA profiling into clinical practice paves the way for personalized orthodontics, where patient-specific molecular signatures inform tailored treatment protocols. Such precision approaches could optimize force application, treatment duration, and the use of adjunctive therapies, thereby enhancing efficacy and minimizing adverse effects. Integration with advanced diagnostic tools—including 3D imaging and artificial intelligence algorithms—may facilitate predictive modeling of treatment responses based on miRNA expression patterns. Ultimately, the convergence of molecular diagnostics, targeted miRNA therapeutics, and digital orthodontics holds promise to revolutionize patient care by delivering highly individualized, efficient, and safer orthodontic interventions (64).

The cost-effectiveness and scalability of miRNA-based therapies

The cost-effectiveness and scalability of miRNA-based therapies are key factors for their integration into routine orthodontic practice. Currently, the synthesis and stabilization of miRNA mimics or inhibitors, along with specialized delivery systems such as

nanoparticles, hydrogels, or functionalized brackets, are still relatively costly compared to conventional orthodontic materials. From a clinical standpoint, miRNA therapies could shorten overall treatment times and reduce the need for corrective procedures, potentially offsetting initial expenses. Regarding scalability, localized delivery methods—such as miRNA-coated brackets or injectable hydrogels—can be incorporated into standard orthodontic workflows without requiring extensive infrastructure changes, making widespread adoption feasible. Additionally, modular delivery platforms that can carry multiple miRNAs could enable personalized, patient-specific treatments, further boosting efficiency and lowering long-term costs. With ongoing development, miRNA-based approaches have the potential to become both practical and economically viable complements to traditional orthodontics, delivering faster, more accurate, and biologically driven tooth movement (65).

Discussion

Understanding the miR-214/miR-206–EphrinA2 axis offers significant translational potential for improving orthodontic treatments and addressing bone metabolic disorders. The ability to specifically modulate these miRNAs, for example, using antagomirs, presents a promising therapeutic approach to enhance osteoblast-driven bone formation. This could accelerate tooth movement, improve post-treatment stability, and benefit patients with impaired bone remodeling. The understanding of how miR-214 and miR-206 influence osteoblast differentiation and bone formation, particularly through their common target EphrinA2, can be crucial for improving fracture healing. Dysregulation of EphrinA2, for example, has been linked to delayed fracture healing and a higher risk of non-union fractures, indicating that therapies targeting this pathway could promote bone regeneration. Elevated levels of miR-214 are associated with bone loss in conditions like osteoporosis, and its inhibition has shown potential to enhance bone formation. Similarly, miR-206 dysregulation contributes to disuse-induced bone loss and sarcopenia-related osteoporosis. Therefore, targeted manipulation of the miR-214/miR-206-EphrinA2 axis could offer new therapeutic strategies for managing conditions with impaired bone remodeling, such as osteoporosis and other bone metabolic disorders (31, 34, 66, 67).

Research Gaps and Future Directions

Off-target effects: A significant concern is that specific miRNAs can regulate numerous genes across various cell lineages. Therefore, extended inhibition or overexpression might inadvertently compromise unrelated signaling cascades, affecting bone homeostasis, angiogenesis, or even systemic organ functionality. For example, excessive repression of miR-214 could disrupt normal bone remodeling in other areas, potentially leading to localized osteopetrosis or delayed bone turnover.

Immune activation: The introduction of miRNA mimics or antagonists, especially via nanoparticles or viral vectors, could be recognized as foreign entities by the immune system, potentially inciting inflammation or hypersensitivity responses. Persistent local immune activation in the periodontal ligament could exacerbate tissue injury or impede orthodontic tooth movement.

Cellular toxicity: If delivery systems accumulate or degrade slowly, cellular toxicity might emerge, affecting fibroblasts, osteoblasts, or osteoclasts. Epigenetic modifications: Enduring epigenetic modifications represent a theoretical hazard, as continuous miRNA modulation might instigate compensatory alterations in gene expression beyond the therapeutic timeframe.

Stability and degradation: miRNAs are prone to rapid degradation in biological fluids, necessitating protective delivery vectors.

Tissue specificity: Achieving targeted delivery to alveolar bone or periodontal ligament without affecting other tissues remains a technical hurdle.

References

1. Nakai Y, Praneetpong N, Ono W, et al. Mechanisms of osteoclastogenesis in orthodontic tooth movement and orthodontically induced tooth root resorption. J Bone Metab. 2023;30(4):297.
2. O'Donnell JS, Long GV, Scolyer RA, et al. Resistance to PD1/PDL1 checkpoint inhibition. Cancer Treat Rev. 2017;52:71-81.
3. Wang X, Qu Z, Zhao S, et al. Wnt/β-catenin signaling pathway: proteins' roles in osteoporosis and cancer diseases and the regulatory effects of natural compounds on osteoporosis. Mol Med. 2024;30(1):193.
4. Sadeghzade A, Jafarian A, Davodpour AH, et al. Oral squamous cell carcinoma pharmacological

treatment; A long non-coding RNAs (long ncRNAs) story. Int J Mol Cell Med. 2025;14(2):736-52.
5. Jiménez-Ortega RF, Ortega-Meléndez AI, Patiño N, et al. The involvement of microRNAs in bone remodeling signaling pathways and their role in the development of osteoporosis. Biology. 2024;13(7):505.
6. Golež A, Ovsenik M, Cankar K. The effect of orthodontic tooth movement on the sensitivity of dental pulp: A systematic review and meta-analysis. Heliyon. 2023;9(4):e14621.
7. Cen X, Pan X, Zhang B, et al. miR-20a-5p contributes to osteogenic differentiation of human dental pulp stem cells by regulating BAMBI and activating the phosphorylation of Smad5 and p38. Stem Cell Res Ther. 2021;12(1):021-02501.
8. Sun Y, Li J, Xie X, et al. Macrophage-osteoclast associations: origin, polarization, and subgroups. Front. Immunol. 2021;12:778078.
9. Bloemen V, Schoenmaker T, De Vries TJ, et al. Direct cell–cell contact between periodontal ligament fibroblasts and osteoclast precursors synergistically increases the expression of genes related to osteoclastogenesis. J. Cell. Physiol. 2010;222(3):565-73.
10. Jeon HH, Yang C-Y, Shin MK, et al. Osteoblast lineage cells and periodontal ligament fibroblasts regulate orthodontic tooth movement that is dependent on Nuclear Factor-kappa B (NF-kB) activation. Angle Orthod. 2021;91(5):664-71.
11. Wang Z, Ren L, Li Z, et al. Impact of Different Cell Types on the Osteogenic Differentiation Process of Mesenchymal Stem Cells. Stem Cells Int. 2025;2025(1):5551222.
12. Zhang R, Oyajobi BO, Harris SE, et al. Wnt/β-catenin signaling activates bone morphogenetic protein 2 expression in osteoblasts. Bone. 2013;52(1):145-56.
13. Jeon HH, Teixeira H, Tsai A. Mechanistic insight into orthodontic tooth movement based on animal studies: a critical review. J Clin Med. 2021;10(8):1733.
14. Sun M, Zhou X, Chen L, et al. The regulatory roles of microRNAs in bone remodeling and perspectives as biomarkers in osteoporosis. BioMed Res Int. 2016;2016(1):1652417.
15. Gao Y, Patil S, Jia J. The development of molecular biology of osteoporosis. Int J Mol Sci. 2021;22(15):8182.
16. Luan X, Zhou X, Trombetta-eSilva J, et al. MicroRNAs and periodontal homeostasis. J Dent Res. 2017;96(5):491-500.
17. Wang X, Guo B, Li Q, et al. miR-214 targets ATF4 to inhibit bone formation. Nat Med. 2013;19(1):93-100.
18. Yang N, Wang L, Liu J, et al. MicroRNA-206 regulates the epithelial-mesenchymal transition and inhibits the invasion and metastasis of prostate cancer cells by targeting Annexin A2. Oncol Lett. 2018;15(6):8295-302.
19. Sadu L, Krishnan RH, Akshaya R, et al. Roles of miR-214 in bone physiology and disease. Biocell. 2023;47(4):751.
20. Zhao C, Sun W, Zhang P, et al. miR-214 promotes osteoclastogenesis by targeting Pten/PI3k/Akt pathway. RNA Biol. 2015;12(3):343-53.
21. Shi K, Lu J, Zhao Y, et al. MicroRNA-214 suppresses osteogenic differentiation of C2C12 myoblast cells by targeting Osterix. Bone. 2013;55(2):487-94.
22. Yu C, Lu Z, Du Y, et al. Inhibition of SFRP1 by microRNA- 206- 3p may be the underlying cause of osteosarcopenia. Biomed Rep. 2025;22(6):103.
23. Zhu S, Chen W, Masson A, et al. Cell signaling and transcriptional regulation of osteoblast lineage commitment, differentiation, bone formation, and homeostasis. Cell Dis. 2024;10(1):71.
24. Kitaura H, Marahleh A, Ohori F, et al. Osteocyte-related cytokines regulate osteoclast formation and bone resorption. Int J Mol Sci. 2020;21(14):5169.
25. Baudet S, Bécret J, Nicol X. Approaches to manipulate Ephrin-A: EphA forward signaling pathway. Pharmaceuticals. 2020;13(7):140.
26. Zhang Y, Li XH, Peng P, et al. RUNX2 Phase Separation Mediates Long- Range Regulation Between Osteoporosis- Susceptibility Variant and XCR1 to Promote Osteoblast Differentiation. Adv Sci. 2025;12(6):2413561.
27. Irie N, Takada Y, Watanabe Y, et al. Bidirectional signaling through ephrinA2-EphA2 enhances osteoclastogenesis and suppresses osteoblastogenesis. J Biol Chem. 2009;284(21):14637-44.
28. Yu X-F, Teng B, Li J-F, et al. Novel Function of Osteocalcin in Chondrocyte Differentiation and

Endochondral Ossification Revealed on a CRISPR/Cas9 bglap–bglap2 Deficiency Mouse Model. Int J Mol Sci. 2024;25(18):9945.
29. Petrovic N, Ergun S. miRNAs as potential treatment targets and treatment options in cancer. Mol Diagn Ther. 2018;22(2):157-68.
30. Zhao C, Sun W, Zhang P, et al. miR-214 promotes osteoclastogenesis by targeting Pten/PI3k/Akt pathway. RNA Biol. 2015;12(3):343-53.
31. Limlawan P, Vacharaksa A. MicroRNA functions in osteogenic differentiation of periodontal ligament stem cells: a scoping review. Front. Oral Health. 2025;6:1423226.
32. Chen Y, Zhang C. Role of noncoding RNAs in orthodontic tooth movement: new insights into periodontium remodeling. 2023;21(1):101.
33. Fang S, Deng Y, Gu P, et al. MicroRNAs regulate bone development and regeneration. Int J Mol Sci. 2015;16(4):8227-53.
34. Kapinas K, Delany AM. MicroRNA biogenesis and regulation of bone remodeling. Arthritis Res Ther. 2011;13(3):220.
35. Ji L, Li X, He S, et al. Regulation of osteoclast-mediated bone resorption by microRNA. Cell Mol Life Seq. 2022;79(6):287.
36. Liu J, Xiao Q, Xiao J, et al. Wnt/β-catenin signalling: function, biological mechanisms, and therapeutic opportunities. Signal Transduct Target Ther. 2022;7(1):3.
37. Amin MM, Trevelyan CJ, Turner NA. MicroRNA-214 in health and disease. Cells. 2021;10(12):3274.
38. Doghish AS, Elballal MS, Elazazy O, et al. miRNAs as potential game-changers in bone diseases: Future medicinal and clinical uses. Pathol. Res. Pract. 2023;245:154440.
39. Lu Z, Wang D, Wang X, et al. MiR-206 regulates the progression of osteoporosis via targeting HDAC4. Eur J Med Res. 2021;26(1):8.
40. Luo J, Pu Q, Wu X. Recent advances of exosomes derived from skeletal muscle and crosstalk with other tissues. Int J Mol Sci. 2024;25(20):10877.
41. Komatsu DE, Duque E, Hadjiargyrou M. MicroRNAs and fracture healing: pre-clinical studies. Bone. 2021;143:115758.
42. Jing D, Hao J, Shen Y, et al. The role of microRNAs in bone remodeling. Int J Oral Sci. 2015;7(3):131-43.
43. Chen N, Sui BD, Hu CH, et al. microRNA-21 Contributes to Orthodontic Tooth Movement. J Dent Res. 2016;95(12):1425-33.
44. Arthur A, Gronthos S. Eph-ephrin signaling mediates cross-talk within the bone microenvironment. Front. Cell Dev. Biol. 2021;9:598612.
45. Giordano G, Tucciarello C, Merlini A, et al. Targeting the EphA2 pathway: could it be the way for bone sarcomas? Cell Commun Signal. 2024;22(1):433.
46. Giannopoulos K, Karikis I, Byrd C, et al. Eph/ephrin-mediated immune modulation: a potential therapeutic target. Front. Immunol. 2025;16:1539567.
47. Lingerak R, Petty A, Shi X, et al. Skeletal Metastasis of Prostate Cancer Is Augmented by Activation of EphA2 Noncanonical Signaling and Ligand-Deficient Bone Microenvironment. bioRxiv. 2024:2024.09. 25.615079.
48. Matsuo K, Otaki N. Bone cell interactions through Eph/ephrin: bone modeling, remodeling and associated diseases. Cell Adh Migr. 2012;6(2):148-56.
49. Xu H, Bai D, Ruest L-B, et al. Expression analysis of α-smooth muscle actin and tenascin-C in the periodontal ligament under orthodontic loading or in vitro culture. Int J Oral Sci. 2015;7(4):232-41.
50. Taulli R, Bersani F, Foglizzo V, et al. The muscle-specific microRNA miR-206 blocks human rhabdomyosarcoma growth in xenotransplanted mice by promoting myogenic differentiation. J Clin Invest. 2009;119(8):2366-78.
51. Cultrera G, Lo Giudice A, Santonocito S. MicroRNA Modulation during Orthodontic Tooth Movement: A Promising Strategy for Novel Diagnostic and Personalized Therapeutic Interventions. Int J Mol Sci. 2022;23(24):15501.
52. Lee SWL, Paoletti C, Campisi M, et al. MicroRNA delivery through nanoparticles. J Control Release. 2019;313:80-95.
53. Li Z, Yu M, Jin S, et al. Stress Distribution and Collagen Remodeling of Periodontal Ligament During Orthodontic Tooth Movement. Front Pharmacol. 2019;10:1263.
54. Hensley AP, McAlinden A. The role of microRNAs in bone development. Bone. 2021;143:115760.

55. Wang P, Zhou Y, Richards AM. Effective tools for RNA-derived therapeutics: siRNA interference or miRNA mimicry. Theranostics. 2021;11(18):8771.
56. Santonocito S, Polizzi A, Palazzo G, et al. The emerging role of microRNA in periodontitis: pathophysiology, clinical potential and future molecular perspectives. Int J Mol Sci. 2021;22(11):5456.
57. Kazanopoulos N, Sideris CD, Xu Y, et al. Identification of Salivary Exosome-Derived miRNAs as Potential Biomarkers of Bone Remodeling During Orthodontic Tooth Movement. Int J Mol Sci. 2025;26(3):1228.
58. Polizzi A, Alibrandi A, Lo Giudice A, et al. Impact of periodontal microRNAs associated with alveolar bone remodeling during orthodontic tooth movement: a randomized clinical trial. J Transl Med. 2024;22(1):1155.
59. Mahmoudi Anzabi R, Shenasa N, Jafarian A, et al. Enhancing Osteogenesis in Dental Implants: The Role of ZNF710-AS1 and miR-146a-5p in Smad Signaling Pathway Activation. Int J Mol Cell Med. 2025;0(0).
60. Castañón-Cortés LG, Bravo-Vázquez LA, Santoyo-Valencia G, et al. Current advances in the development of microRNA-integrated tissue engineering strategies: a cornerstone of regenerative medicine. Front Bioeng Biotechnol. 2024;12:1484151.
61. Dubey AK, Mostafavi E. Biomaterials-mediated CRISPR/Cas9 delivery: recent challenges and opportunities in gene therapy. Front. Chem. 2023;11:1259435.
62. Wang S, Liu Y, Fang D, et al. The miniature pig: a useful large animal model for dental and orofacial research. Oral Dis. 2007;13(6):530-7.
63. Tarantal AF, Noctor SC, Hartigan-O'Connor DJ. Nonhuman Primates in Translational Research. Annu Rev Anim Biosci. 2022;10:441-68.
64. Farran WH, Tarabzouni KW, Almuqati TA, et al. The growing role of genetics in orthodontic diagnosis and treatment planning. J Health Sci. 2024;4:515-20.
65. Dasgupta I, Chatterjee A. Recent Advances in miRNA Delivery Systems. Methods Protoc. 2021;4(1):10.
66. Yuan FL, Wu QY, Miao ZN, et al. Osteoclast-Derived Extracellular Vesicles: Novel Regulators of Osteoclastogenesis and Osteoclast-Osteoblasts Communication in Bone Remodeling. Front Physiol. 2018;9:628.
67. Chen B, Zhang Y, Liu O. MicroRNAs in maxillofacial bone modeling and remodeling: implications for malocclusion development and orthodontic treatment. Front Cell Dev Biol. 2024;12:1355312.