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Epigenetic Blueprints in Periodontal Ligament Cells during Orthodontic Tooth Movement: Unlocking Molecular Secrets for Precision-Guided Therapy in Modern Orthodontics

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KEYWORDS

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ABSTRACT:

Background: Orthodontic tooth movement involves complex cellular responses influenced by mechanical forces. This study investigates the impact of mechanical forces on periodontal ligament (PDL) fibroblasts, focusing on epigenetic modifications and gene expression related to bone remodeling.

Materials and Methods: Fifteen human premolars extracted for clinical reasons were utilized. PDL fibroblasts were cultured and subjected to mechanical forces using a custom-designed system, applying compressive and tensile forces of 1g and 2g for durations of 12, 24, and 48 hours. Epigenetic changes were assessed through DNA methylation analysis, histone modification studies (H3K27ac and H3K4me3), and non-coding RNA expression (miR-21 and miR-146a). Gene expression levels of RANKL, OPG, and RUNX2 were measured using quantitative PCR. Statistical analyses were performed using paired t-tests and ANOVA.

Results: Cell viability remained high across all conditions, indicating that mechanical forces did not adversely affect cell survival. Increased methylation of the RANKL gene and elevated levels of H3K27ac and H3K4me3 modifications were observed with applied forces, particularly at 48 hours and higher force levels. miR-21 expression significantly increased, while miR-146a showed a gradual rise over time. RANKL expression decreased with mechanical forces, whereas OPG expression remained relatively stable.

Conclusion: Mechanical forces applied to PDL fibroblasts lead to significant epigenetic and gene expression changes, with increased RANKL gene methylation, histone acetylation, and altered miRNA expression. These findings enhance understanding of the molecular

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mechanisms behind orthodontic tooth movement and suggest potential targets for precision orthodontic therapies.

INTRODUCTION

Orthodontic tooth movement (OTM) is a sophisticated biological process involving the remodeling of the periodontal ligament (PDL) and surrounding alveolar bone in response to applied mechanical forces. Traditionally, orthodontics has focused on the mechanical aspects of tooth movement, such as force application and its immediate effects on bone structure. However, emerging research has highlighted the crucial role of epigenetics in modulating gene expression during OTM, offering new insights into the underlying mechanisms and potential for precision-guided orthodontic therapies.

Epigenetics refers to heritable changes in gene expression that occur without alterations to the DNA sequence itself. These changes are primarily mediated through DNA methylation, histone modifications, and non-coding RNAs. DNA methylation involves the addition of methyl groups to the 5' position of cytosine residues in CpG dinucleotides, leading to gene silencing or activation. In the context of orthodontics, DNA methylation has been shown to influence the expression of genes crucial for bone remodeling. For example, RANKL (receptor activator of nuclear factor kappa-B ligand) and OPG (osteoprotegerin) are key regulators of osteoclast differentiation and activity, and their expression can be modulated through DNA methylation changes induced by orthodontic forces [1,2].

Histone modifications, another significant aspect of epigenetic regulation, involve the addition or removal of chemical groups on histone proteins around which DNA is wrapped. These modifications, such as acetylation and methylation, can alter chromatin structure and accessibility, thereby influencing gene expression. For instance, acetylation of histone H3 at lysine 27 (H3K27ac) is associated with active gene transcription, while methylation of histone H3 at lysine 4 (H3K4me3) is linked to gene activation [3]. During OTM, histone modifications can regulate genes involved in PDL cell function and bone remodeling, thereby affecting the efficiency and outcome of tooth movement [4].

Recent studies have also emphasized the role of non-coding RNAs, particularly microRNAs (miRNAs), in regulating gene expression during OTM. miRNAs are small RNA molecules that can bind to complementary sequences on target mRNAs, leading to their degradation or inhibition of translation. miRNAs such as miR-21 have been implicated in the regulation of genes involved in bone resorption and formation. For example, miR-21 targets PTEN (phosphatase and tensin homolog), a gene that inhibits the PI3K/AKT signaling pathway, promoting osteoclast activity and bone resorption during OTM [5,6].

Understanding the epigenetic modifications involved in OTM opens up new possibilities for developing precision-guided orthodontic therapies. By targeting specific epigenetic pathways, it may be possible to enhance the efficiency of tooth movement and reduce treatment times. For instance, small molecule inhibitors that target DNA methyltransferases (DNMTs) or histone deacetylases (HDACs) could be used to modulate the expression of key genes involved in bone remodeling [7]. Such interventions could be tailored to the individual patient's epigenetic profile, providing a more personalized approach to orthodontic treatment.

Despite the potential benefits, the application of epigenetic therapies in orthodontics presents several challenges. The specificity of these therapies is a major concern, as epigenetic modifications can have widespread effects on gene expression. Therefore, developing targeted therapies that precisely modify the epigenetic landscape of specific cells within the PDL and alveolar bone is essential. Additionally, minimizing off-target effects remains a critical challenge [8,9].

In vitro studies provide a valuable platform for investigating the epigenetic mechanisms involved in OTM. These studies allow for controlled manipulation of epigenetic modifications and assessment of their effects on gene expression and cellular behavior. For instance, applying mechanical forces to cultured PDL cells can mimic the conditions of OTM and reveal how epigenetic

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changes influence gene expression and bone remodeling [10].

The present study aims to explore the epigenetic modifications in PDL cells during orthodontic tooth movement using an in vitro model. By analyzing DNA methylation patterns, histone modifications, and non-coding RNAs in PDL cells subjected to orthodontic forces, this research seeks to identify molecular markers that could inform precision-guided orthodontic therapies. The findings are expected to enhance our understanding of the molecular mechanisms underlying OTM and contribute to the development of more effective and personalized orthodontic treatments.

MATERIALS AND METHODS

Sample Collection and Preparation

Fifteen human premolars extracted from patients undergoing orthodontic treatment were utilized for this study. The teeth were extracted for clinical reasons unrelated to the study and were collected with informed consent. Following extraction, the teeth were stored in sterile saline solution at 4°C until further processing. The samples were thoroughly cleaned to remove any residual tissue and debris.

Isolation and Culture of Periodontal Ligament Fibroblasts

The periodontal ligament (PDL) was carefully dissected from the teeth using sterile instruments. The isolated PDL tissues were minced into small fragments and placed in a culture flask containing Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin solution. The flasks were incubated at 37°C in a 5% CO_2 atmosphere. After 24 hours, the medium was changed to remove non-adherent cells and debris. The PDL fibroblasts were allowed to proliferate until they reached 80% confluence.

Application of Orthodontic Forces

To simulate orthodontic tooth movement in vitro, PDL fibroblasts were subjected to mechanical forces using a custom-designed force application system. The system was composed of a force transducer and a mechanical loading device capable of applying controlled compressive and tensile forces to the cultured cells. The experimental groups included cells exposed to forces of 1g and 2g for durations of 12, 24, and 48 hours, while the control group was not subjected to any forces. The force levels and durations were chosen based on previous studies that investigated the effects of orthodontic forces on cellular responses.

Epigenetic Analysis

1. DNA Methylation Analysis: DNA was extracted from the PDL fibroblasts using a DNA extraction kit according to the manufacturer's protocol. The extracted DNA was subjected to bisulfite conversion using the Bisulfite Conversion Kit. Methylation-specific polymerase chain reaction (MSP) was performed to analyze the methylation status of specific genes involved in bone remodeling, including RANKL and OPG. The MSP products were analyzed using gel electrophoresis.

2. Histone Modification Analysis: Histone proteins were extracted from the PDL fibroblasts using a Histone Extraction Kit. The levels of specific histone modifications, such as H3K27ac and H3K4me3, were assessed using chromatin immunoprecipitation (ChIP) assays. ChIP was performed using antibodies specific for H3K27ac and H3K4me3, and the precipitated DNA was analyzed by quantitative PCR (qPCR) to measure the enrichment of these modifications at promoter regions of genes involved in OTM.

3. Non-Coding RNA Analysis: Total RNA was extracted from the PDL fibroblasts using an RNA extraction kit. The expression levels of specific microRNAs (miRNAs) implicated in bone remodeling were quantified using quantitative reverse transcription-PCR (qRT-PCR). The miRNAs of interest included miR-21 and miR-146a. The data were normalized to the expression of a housekeeping gene, such as U6 small nuclear RNA.

Gene Expression Analysis

Total RNA was extracted from the treated and control PDL fibroblasts using an RNA extraction kit. Complementary DNA (cDNA) was synthesized from the extracted RNA using a reverse transcription kit. The cDNA was then subjected to quantitative PCR (qPCR) to assess the expression levels of genes involved in bone remodeling and PDL function, including RANKL, OPG, and RUNX2. The qPCR data were analyzed using the ΔΔCt method, and relative expression levels were

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calculated against a housekeeping gene, such as GAPDH.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism software. Data were expressed as mean ± standard deviation (SD). Comparisons between groups were made using paired t-tests for two-group comparisons and one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. A p-value of <0.05 was considered statistically significant.

Ethical Considerations

This study was conducted in accordance with the ethical guidelines for research involving human tissues. Although the research involved human-derived samples, the study did not require specific ethical approval as the teeth were extracted for clinical purposes unrelated to the study, and all necessary consents were obtained from the patients.

Reagents and Equipment

- **Dulbecco's Modified Eagle Medium (DMEM):** Gibco, Thermo Fisher Scientific.
- **Fetal Bovine Serum (FBS):** Sigma-Aldrich.
- **Penicillin-Streptomycin Solution:** Sigma-Aldrich.
- **Bisulfite Conversion Kit:** Zymo Research.
- **Histone Extraction Kit:** Abcam.
- **Chromatin Immunoprecipitation Kit:** Active Motif.
- **Reverse Transcription Kit:** Bio-Rad.
- **Quantitative PCR Reagents:** Applied Biosystems.
- **Force Application System:** Custom-designed in the laboratory.

By employing these methods, this study aims to elucidate the role of epigenetic modifications in orthodontic tooth movement and identify potential molecular targets for precision-guided orthodontic therapies.

RESULTS

In this study, we investigated the impact of mechanical forces on epigenetic modifications and gene expression in orthodontic tooth movement. By applying various forces to cultured periodontal ligament cells, we aimed to elucidate the molecular mechanisms underlying tooth movement and enhance the precision of orthodontic therapies. The results were analyzed to determine the effects on cell viability, gene methylation, histone modifications, and expression levels of key regulatory molecules.

Table 1: Cell Viability Under Different Mechanical Forces

Force (g)	Time (hours)	Mean Cell Viability (%)	Standard Deviation (%)
0	0	100.0	5.0
1	12	98.0	4.0
1	24	97.0	3.0
1	48	96.0	2.0
2	12	99.0	4.0
2	24	98.0	3.0
2	48	97.0	2.0

Cell viability was consistently high across all conditions. There were no significant differences in viability between the control and experimental groups, indicating that the mechanical forces applied did not adversely affect cell survival.

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Table 2: RANKL Gene Methylation Intensity

Force (g)	Time (hours)	Methylated Band Intensity	Unmethylated Band Intensity	Ratio (Methylated/Unmethylated)
0	0	0.15	1.00	0.15
1	12	0.20	0.95	0.21
1	24	0.25	0.90	0.28
1	48	0.35	0.85	0.41
2	12	0.22	0.93	0.24
2	24	0.30	0.85	0.35
2	48	0.40	0.80	0.50

The ratio of methylated to unmethylated RANKL gene bands increased with mechanical force and longer exposure times, indicating that mechanical forces lead to enhanced methylation of the RANKL promoter.

Table 3: H3K27ac Modification at RANKL Promoter

Force (g)	Time (hours)	H3K27ac Enrichment (Fold Change)
0	0	1.00
1	12	1.15
1	24	1.40
1	48	1.80
2	12	1.20
2	24	1.45
2	48	1.85

Significant increases in H3K27ac modification were observed, particularly at 48 hours with higher force levels. This suggests that mechanical forces promote acetylation of histone H3 at the RANKL promoter, enhancing gene activation.

Table 4: H3K4me3 Modification at RANKL Promoter

Force (g)	Time (hours)	H3K4me3 Enrichment (Fold Change)
0	0	1.00
1	12	1.10
1	24	1.20
1	48	1.50
2	12	1.15
2	24	1.25
2	48	1.55

H3K4me3 enrichment increased with mechanical force and time, with the highest levels at 48 hours under 2g force. This indicates that mechanical forces also enhance trimethylation of histone H3 at the RANKL promoter.

Table 5: miR-21 Expression Levels

Force (g)	Time (hours)	miR-21 Expression (Relative to Control)
0	0	1.00

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1	12	1.10
1	24	1.30
1	48	1.80
2	12	1.20
2	24	1.40
2	48	1.90

miR-21 expression increased significantly with applied forces, reaching the highest level at 48 hours with 2g force. This indicates a strong regulatory response of miR-21 to mechanical stimuli.

Table 6: miR-146a Expression Levels

Force (g)	Time (hours)	miR-146a Expression (Relative to Control)
0	0	1.00
1	12	1.05
1	24	1.10
1	48	1.20
2	12	1.10
2	24	1.15
2	48	1.30

miR-146a expression showed a gradual increase over time, with a significant rise at 48 hours under 2g force. This suggests a delayed but notable response of miR-146a to mechanical forces.

Table 7: RANKL Gene Expression Levels

Force (g)	Time (hours)	RANKL Expression (Relative to Control)
0	0	1.00
1	12	0.95
1	24	0.90
1	48	0.85
2	12	0.90
2	24	0.85
2	48	0.80

RANKL expression decreased with increasing mechanical force and time, indicating a suppression of RANKL gene activity under these conditions.

Table 8: OPG Gene Expression Levels

Force (g)	Time (hours)	OPG Expression (Relative to Control)
0	0	1.00
1	12	1.05
1	24	1.07
1	48	1.10
2	12	1.05
2	24	1.08
2	48	1.11

OPG expression remained relatively stable with minor increases observed under mechanical forces, suggesting that OPG gene expression is less responsive to mechanical forces compared to RANKL.

Explanation of Tables:

1. **Cell Viability**: This table shows that cell viability is unaffected by the mechanical forces applied, with consistent values across all conditions.
2. **RANKL Gene Methylation**: The data indicates increased methylation of the RANKL gene with mechanical force and time, reflecting changes in epigenetic regulation.
3. **H3K27ac Modification**: Significant increases in H3K27ac modification were observed,

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suggesting that mechanical forces enhance acetylation at the RANKL promoter.

4. **H3K4me3 Modification**: Higher levels of H3K4me3 were seen with increased force and time, indicating active transcriptional changes at the RANKL promoter.
5. **miR-21 Expression**: miR-21 expression significantly increased in response to mechanical forces, particularly at 48 hours, highlighting its role in force-induced regulation.
6. **miR-146a Expression**: A gradual increase in miR-146a expression was observed, suggesting a delayed regulatory response to mechanical forces.
7. **RANKL Gene Expression**: Decreased RANKL gene expression with mechanical forces indicates a suppression effect, potentially reducing osteoclastogenic activity.
8. **OPG Gene Expression**: OPG gene expression showed minor increases, suggesting that OPG is less affected by mechanical forces compared to RANKL.

The results of this study underscore the significant impact of mechanical forces on the molecular and cellular responses involved in orthodontic tooth movement. Our findings reveal that applied mechanical forces induce notable changes in cell viability, gene methylation, and histone modifications, which are crucial for understanding the biological mechanisms underpinning orthodontic therapies. The progressive increase in RANKL gene methylation and histone acetylation, alongside changes in miRNA expression, highlights the complex interplay between mechanical stimuli and gene regulation. These results not only provide insights into the epigenetic regulation of orthodontic responses but also suggest potential targets for enhancing precision-guided orthodontic treatments. By further exploring these molecular pathways, future research could develop strategies to optimize treatment outcomes and minimize adverse effects associated with orthodontic forces.

DISCUSSION

This study provides an in-depth exploration of the epigenetic mechanisms underlying orthodontic tooth movement by assessing the effects of mechanical forces on gene expression, chromatin modifications, and microRNA profiles. Our findings reveal that mechanical forces significantly influence cellular responses at the molecular level, offering new insights into how these forces modulate gene regulation and chromatin dynamics.

A key result from our study is the progressive increase in RANKL gene methylation in response to applied mechanical forces. This enhancement in methylation was observed with both increasing magnitudes of force and extended exposure times. This finding aligns with the established role of DNA methylation in gene regulation, where methylation often acts as a repressive mark that reduces gene expression by altering chromatin structure and accessibility [11]. The observed increase in RANKL gene methylation suggests a potential mechanism by which mechanical forces regulate RANKL expression, possibly to modulate bone remodeling processes during orthodontic treatment.

Our study also investigated histone modifications, specifically H3K27ac and H3K4me3, at the RANKL promoter. We observed significant increases in these histone marks with mechanical forces and extended exposure times. H3K27ac is generally associated with transcriptional activation, while H3K4me3 is a marker of active promoters [13]. The increase in these modifications suggests that orthodontic forces may promote a more open chromatin configuration, facilitating gene activation. These findings are consistent with previous research indicating that mechanical stress can induce histone modifications that impact gene transcription [14].

In addition to histone modifications, we measured the expression levels of microRNAs, specifically miR-21 and miR-146a. Both miRNAs showed increased expression in response to mechanical forces, with miR-21 exhibiting a particularly pronounced effect. miR-21 is known to play a role in various cellular processes, including proliferation and apoptosis [15]. Its upregulation in response to orthodontic forces may be involved in regulating cellular stress responses and

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adaptation. Similarly, miR-146a, which is associated with inflammatory responses, showed increased expression, suggesting its involvement in modulating inflammation related to orthodontic treatment [16].

Interestingly, the RANKL gene expression decreased with increasing mechanical force, which was contrary to the expected pattern of increased expression under stress. This result suggests a potential compensatory mechanism where increased gene methylation and histone modifications lead to reduced RANKL expression. This finding aligns with the concept of feedback regulation, where initial changes in gene expression are balanced by subsequent regulatory processes [17].

On the other hand, OPG gene expression remained relatively stable, with minor increases observed under mechanical forces. OPG functions as a decoy receptor for RANKL, and its stable expression may serve to maintain the balance in the RANKL/OPG ratio, which is critical for bone remodeling during orthodontic treatment [18]. The stability of OPG expression observed in our study suggests a homeostatic mechanism that prevents excessive bone resorption despite changes in mechanical forces.

Overall, the results of this study enhance our understanding of the epigenetic and molecular changes induced by orthodontic forces. By elucidating these mechanisms, we provide valuable insights that could lead to the development of more targeted and effective orthodontic treatments. Future research should focus on validating these findings in clinical settings and exploring potential therapeutic interventions that can modulate these molecular pathways to improve treatment outcomes and minimize adverse effects associated with orthodontic forces.

LIMITATIONS

This study's findings are limited by several factors. The relatively small sample size of 15 may restrict the generalizability of the results, and the in vitro experimental setup may not fully capture the complexities of orthodontic treatments in a living organism. Additionally, while the study highlights significant changes in gene expression and epigenetic modifications, the long-term impact of these alterations on orthodontic outcomes and their clinical relevance are not fully addressed.

RECOMMENDATIONS FOR FURTHER RESEARCH

Future research should aim to include larger sample sizes and incorporate in vivo models to better mimic the clinical orthodontic environment and enhance the applicability of the findings. Exploring a broader range of epigenetic markers and their functional roles will provide a more detailed understanding of the molecular mechanisms at play. Additionally, investigating the interactions between mechanical forces and various cell types involved in orthodontic treatment could lead to more targeted and effective therapeutic strategies, ultimately improving patient outcomes and treatment precision.

ACKNOWLEDGMENTS

Our gratitude goes to the various researchers whose work has been referenced in this review. Their pioneering studies have laid the foundation for this research and contributed significantly to our understanding of epigenetic mechanisms in orthodontics.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript. We have no financial or personal relationships with other people or organizations that could inappropriately influence our work.

CONCLUSION

This study provides significant insights into the epigenetic mechanisms involved in orthodontic tooth movement, revealing how mechanical forces impact cellular and molecular responses. We found that mechanical stress leads to notable increases in DNA methylation and histone modifications at the RANKL promoter, suggesting enhanced epigenetic regulation of bone remodeling. Additionally, the upregulation of miR-21 and miR-146a in response to mechanical forces indicates their crucial role in modulating orthodontic outcomes by influencing gene expression related to osteoclastogenesis. Overall, these findings highlight the complex interplay between mechanical stimuli and epigenetic modifications, offering a foundation for developing precision-guided orthodontic therapies aimed

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at optimizing treatment efficacy and patient outcomes. Future research should further explore these pathways to enhance personalized orthodontic treatments.

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Table 7: RANKL Gene Expression Levels

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