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LABORATORY ASSESSMENT OF CRISPR-MEDIATED MODULATION OF OSTEOBLASTIC AND OSTEOCLASTIC GENE EXPRESSION UNDER SIMULATED ORTHODONTIC FORCE

Alam M.K.[1,2,3*], Almohammed Y.E.M.[1], Hajeer M.Y.[4], Alanazi A.W.N.[1], Alanazi F.S.A.[1], Alnawmasi Y.M.F.[1]

[1.] Preventive Dentistry Department, College of Dentistry, Jouf University, Sakaka, Saudi Arabia
[2.] Department of Public Health, Faculty of Allied Health Sciences, Daffodil International University, Dhaka, Bangladesh
[3.] Dental Research Cell, Saveetha Institute of Medical and Technical Sciences, Saveetha Dental College and Hospitals, Chennai, India
[4.] Department of Orthodontics, Faculty of Dentistry, University of Damascus, Damascus, Syria

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Abstract

Introduction: *Orthodontic tooth movement is governed by coordinated bone resorption and formation mediated primarily through the Receptor Activator of Nuclear Factor-κB Ligand – Osteoprotegerin signaling axis. Although mechanical force initiates this process, the biological rate of remodeling remains a limiting factor. CRISPR-based transcriptional activation presents a novel strategy to amplify force-induced molecular responses.*

Material and Methods: *Human periodontal ligament stem cells were exposed to simulated compressive orthodontic force (2 g/cm²) and subjected to CRISPR-dCas9-VPR–mediated activation of the TNFSF11 (RANKL) promoter. Samples were divided into control, force-only, scramble control, and CRISPR-RANKL groups. Cell viability was assessed using CCK-8 assay, while gene and protein expression of RANKL, OPG, and RUNX2 were evaluated using RT-qPCR and ELISA.*

Results: *Cell viability exceeded 90% across all groups, indicating no cytotoxic effects. CRISPR-mediated activation significantly enhanced RANKL expression under compressive force, producing a marked increase in the RANKL/OPG ratio compared with force alone ($p < 0.001$). RUNX2 expression was reduced under compression, consistent with osteoclastic dominance, and was unaffected by CRISPR modulation.*

Conclusion: *CRISPR-dCas9-VPR–based activation of RANKL synergistically augments mechanical force–induced osteoclastic signaling in periodontal ligament stem cells. This proof-of-concept study highlights the potential of epigenetic modulation as a precision approach for biologically accelerating orthodontic tooth movement.*

Keywords: *CRISPR-Cas9; Orthodontic tooth movement; RANKL; Osteoclastogenesis; Periodontal ligament stem cells; Gene activation*

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Address for Correspondence:
Mohammad Khursheed Alam; PhD, Prof.
Department preventive dentistry, College of Dentistry Jouf University, Sakaka 72345, Saudi Arabia
Tel.: +966 535602339
E-mail: mkalam@ju.edu.sa