Nanovibrational stimulation for mesenchymal stromal cell osteogenesis: investigating the relationship between osteogenesis and inflammation

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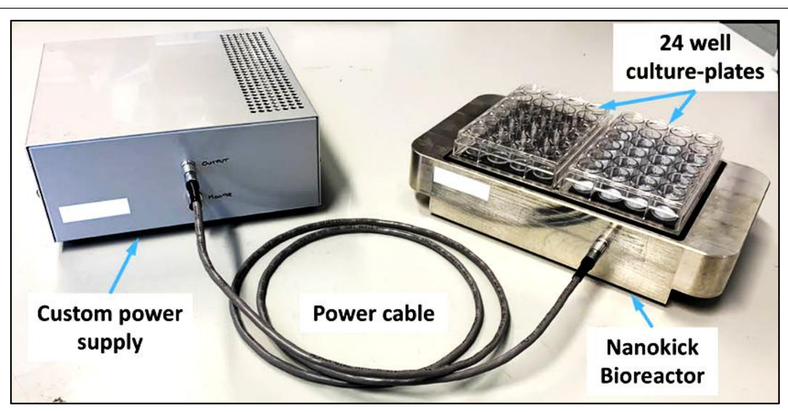
Introduction

Bone anomalies have posed a significant global burden, underscoring the need for deeper insights into bone biomechanics, pathophysiology, and regenerative strategies [1]. Conventional bone tissue engineering relies on exogenous agents, such as growth factors (e.g., BMP2) and corticosteroids (e.g., dexamethasone), to promote osteogenesis, however, limitations exist, including a lack of differentiation specificity, safety concerns and side effects [2]. We have developed a nanovibrational bioreactor capable of inducing mesenchymal stem cell (MSC) osteogenesis at a 30 nm amplitude (NK30, 1 kHz) without any chemical supplementation. Preliminary data suggest enhanced osteogenic differentiation and optimal reactive oxygen species (ROS) and inflammatory signalling at 90 nm (NK90). Considering this, we aim to investigate high-amplitude nanovibration (100 nm, NK100, 1 kHz) to elucidate its effects on MSC osteogenesis, redox balance, and inflammatory pathways [3].

Aims and objectives

The study aims to investigate the effects of high amplitude nanostimulation [100 nm (NK100, 1 kHz)] as compared to the standard 30 nm, 1 kHz on:

1. The expression profiles of osteogenic differentiation, inflammation markers.
2. Phenotype change, followed by emphasis on respiration-related ROS and inflammation.



Methodology

Human Bone marrow-derived primary MSCs (hBMSCs) were cultured at NK30 and NK100 with osteogenic media (OGM), basal control, and stress conditions (10 µM TBHP, 0.1 µM Doxorubicin) for 4 and 6 weeks. In-cell western was performed to quantify phospho-p38/p38 ratio, osteopontin (OPN), and osteocalcin (OCN) protein expression. Late-stage matrix mineralization was determined by Von Kossa staining. Flow cytometry was used to analyse total ROS production, while in-cell western assessed protein expression of glutathione reductase (GSR), superoxide dismutase 1 (SOD1), and catalase. DNA damage was determined based on the number of YH2AX-foci using immunostaining. Secretory profiling of inflammatory cytokines (IL-6, TNF-α, IL-8) was performed using ELISA.

Results

(1) Osteogenic differentiation marker expression

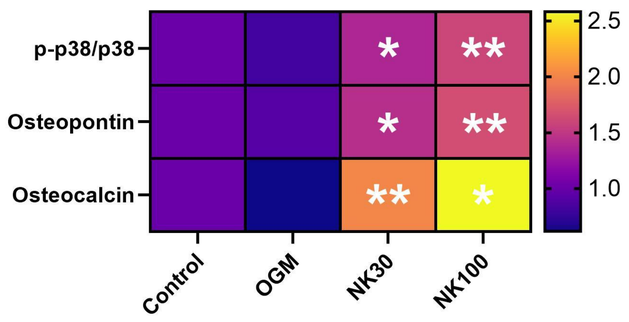


Fig. 1. Protein expression levels of late osteogenic differentiation markers. hBMSCs grown at NK30, NK100, OGM, and control were examined for p-p38/p38, OPN and OCN protein expression at 42 days. Unpaired t-test was performed relative to control for statistical analysis. (Mean±SEM, N=3) *p≤0.05 and **p≤0.01 statistical significances were observed.

(2) Late-stage matrix mineralisation

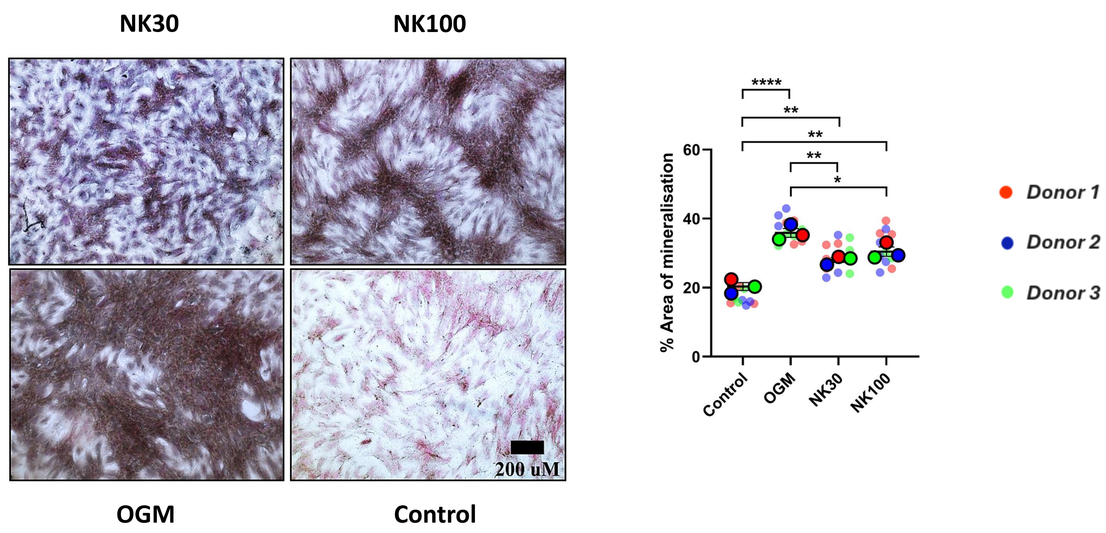


Fig. 2. Quantification of late-stage matrix mineralisation. Both NK30 and NK100 exhibited considerable deposition of hydroxyapatite or calcium phosphate minerals at 42 days, as indicated by an increased % area of mineralisation. One-WAY ANOVA was performed for statistical analysis. (Mean±SEM, N=3) *p≤0.05, **p≤0.01 and ****p≤0.0001 statistical significances were observed.

(3) Total ROS production

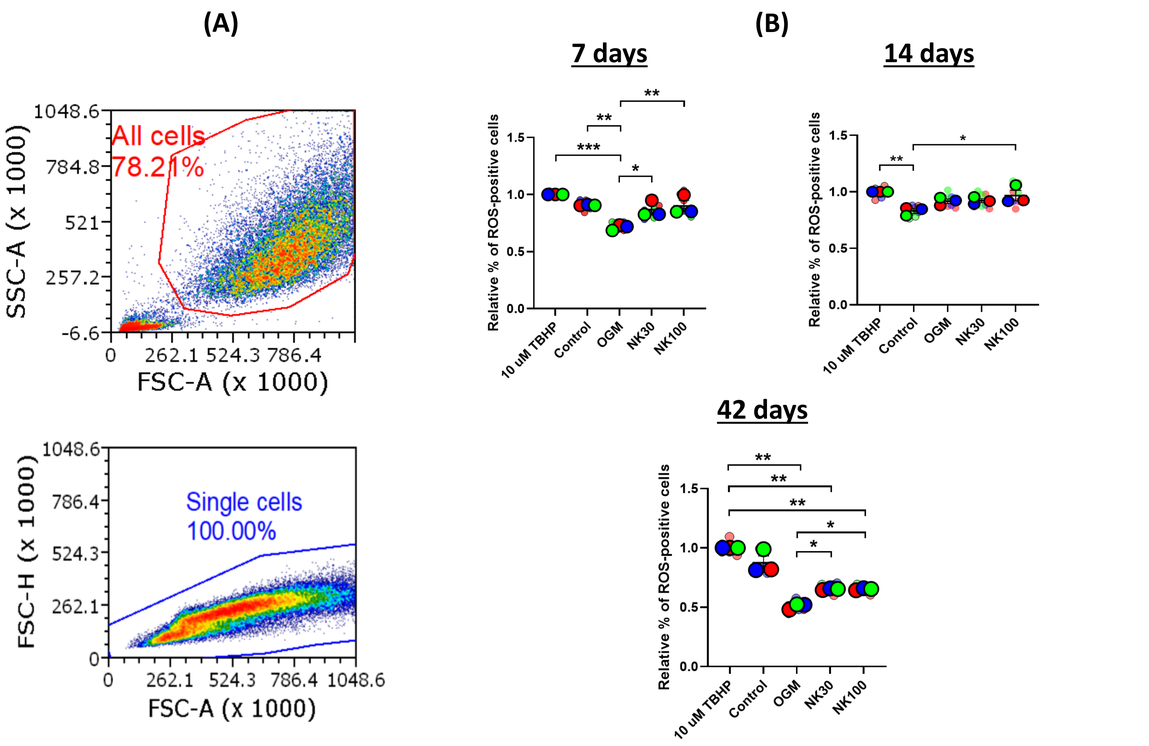


Fig. 3. Comparative estimation of ROS-positive cells. hBMSCs grown at NK30, NK100, OGM, and control were analysed for total ROS production levels at 7, 14 and 42 days. Cells treated with TBHP served as a positive control. **(A)** Representative FSC-A (x-axis) versus SSC-A (y-axis) plot showing the total viable cell population, followed by FSC-A (x-axis) versus FSC-H (y-axis) for doublet exclusion. **(B)** Comparison of conditions based on the % of ROS-positive cells normalised to TBHP. One-Way ANOVA was performed for statistical analysis. (Mean±SEM, N=3) *p≤0.05, **p≤0.01 and ***p≤0.001 statistical significances were observed.

(4) Antioxidation marker expression

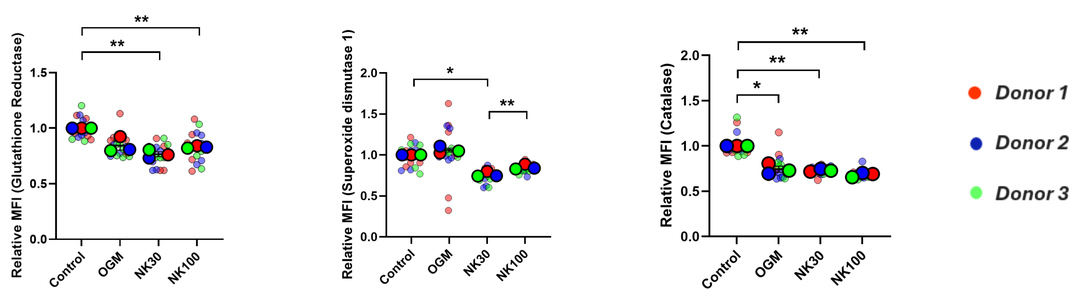


Fig. 4. Protein expression levels of antioxidation markers. (A) hBMSCs grown at NK30, NK100, OGM, and control were examined for glutathione reductase (GSR), superoxide dismutase 1 (SOD1), and catalase at 7 days. One-WAY ANOVA was performed for statistical analysis. (Mean±SEM, N=3) *p≤0.05 and **p≤0.01 statistical significances were observed.

(5) DNA damage (ϒH2AX formation)

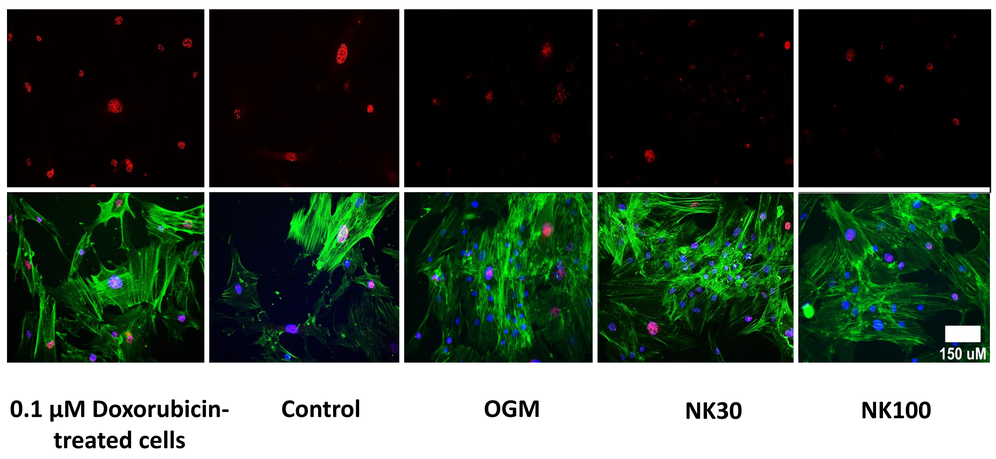


Fig. 5. Assessment of DNA damage via ϒH2AX formation. Representative immunofluorescence images showing ϒH2AX foci (red), cytoskeleton (green) and nuclei (blue) in hBMSCs grown at NK30, NK100, OGM, and control for 7 days. Cells treated with 0.1 µM doxorubicin were used as a positive control for DNA damage. Decreased ϒH2AX foci indicates less DNA damage. Kruskal-Wallis test was performed for statistical analysis. (Mean±SEM, N=3) *p≤0.05, **p≤0.01, ***p≤0.001 and ****p≤0.0001 statistical significances were observed.

(6) Pro-inflammatory cytokine profiling

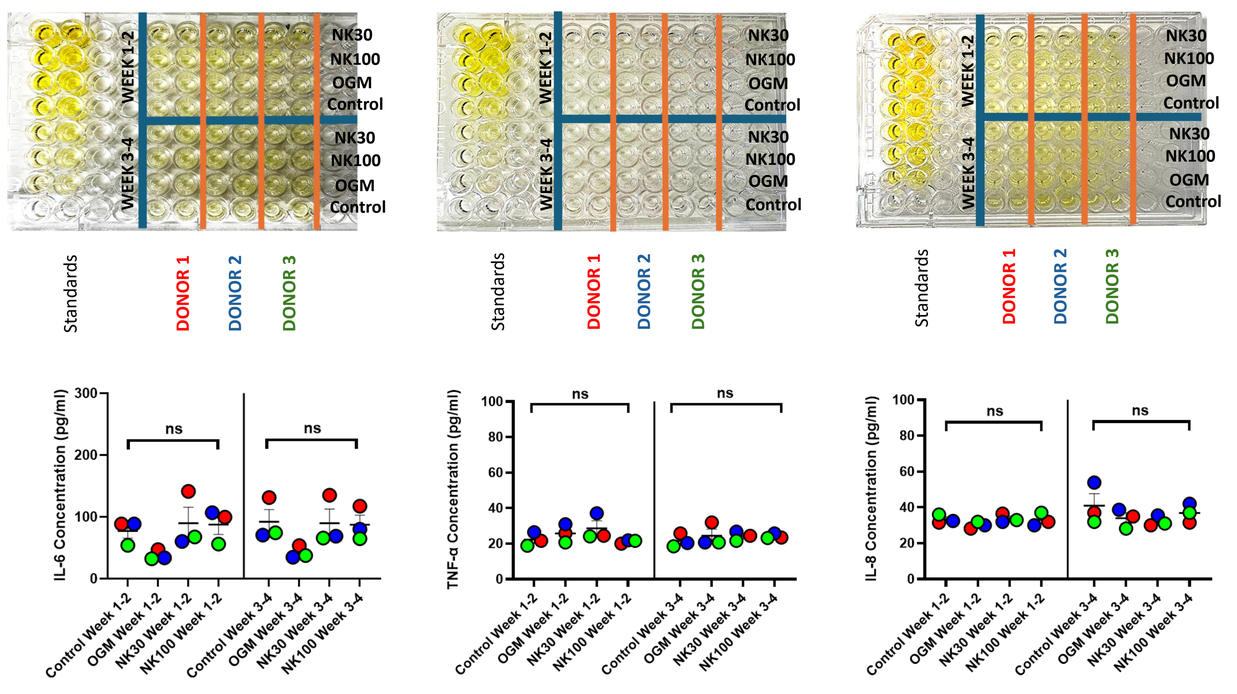


Fig. 6. Proinflammatory cytokine profiling. hBMSCs grown at NK30, NK100, OGM, and control were analysed for the secreted levels of (A) IL-6, (B) TNFα, and **(C)** IL-8 at 1-2 and 3-4 weeks. Comparison of conditions is based on cytokine concentration (pg/ml). Kruskal-Wallis test was performed for statistical analysis. (Mean±SEM, N=3) No statistical significance was observed.

Conclusion and future work

Our findings suggest that high-amplitude nanostimulation may promote enhanced MSC osteogenesis, likely under eustress or mild physiological stress and optimal inflammatory conditions. This is supported by considerable late-stage matrix mineralisation, optimal total ROS production, and non-significant secretion of pro-inflammatory factors, which is further reflected by the decreased expression of antioxidant markers. However, to substantiate these outcomes, we aim to employ RNA sequencing to investigate relevant signalling pathways, along with collagen gel contraction assay and recipher assay to assess oxygen consumption rate (OCR).

References

[1] Shen Y *et al.* Front. Endocrinol. 13: 882241, 2022.

[2] Childs PG *et al.* Biochem. J. 477: 3349–3366, 2020.

[3] Orapiriyakul W *et al*. A.C.S. Nano. 14: 10027–10044, 2020.

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