

Continuous Low-Intensity Ultrasound Reprograms Fibronectin Fragment-Activated Macrophages Toward a Reparative Phenotype: Implications for Early Post-Traumatic Osteoarthritis

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INTRODUCTION: Macrophages play a central role in joint homeostasis and post-traumatic osteoarthritis (PTOA) progression or resolution. Following cartilage injury, fibronectin fragments (Fnfs) from extracellular matrix degradation polarize macrophages toward a pro-inflammatory M1 phenotype, contributing to synovial inflammation and cartilage breakdown. Continuous low-intensity ultrasound (cLIUS), a biophysical modality, has been proposed as a non-pharmacologic therapeutic to modulate inflammatory responses. We hypothesize that cLIUS would attenuate Fnfs-induced inflammatory macrophage activation and promote a reparative M2-like phenotype.

METHODS: THP-1-derived macrophages were encapsulated in 3D hydrogels and polarized to M1 with Fnfs (25 µg/mL, 72 h). Experimental groups received cLIUS stimulation (5 MHz, 14 kPa, 20 min ×4/day, 3 days). Viability was assessed by Live/Dead confocal imaging. RNA was isolated (n=3 replicates/group) for RNA-seq and qRT-PCR validation. Differential expression (DESeq2) and differential clustering analyses were performed, followed by Ingenuity Pathway and Reactome enrichment analyses. Statistical analyses employed one-way ANOVA with Sidak's post-hoc test.

RESULTS: cLIUS maintained >95% cell viability while reprogramming Fnfs-activated macrophages. RNA-seq identified 149 genes upregulated and 25 downregulated with cLIUS (padj<0.05). Pro-inflammatory markers (IL1B, TNF, CCL3) were significantly downregulated, while reparative and M2-associated genes (PTGDS, TGFBI, GRN, ORM1, CD163) were upregulated. Differential clustering revealed extensive transcriptional network rewiring, with enhanced pathways for extracellular matrix organization, tissue remodeling, and resolution of inflammation, alongside suppression of TNF and NF-κB signaling. qRT-PCR confirmed downregulation of IL1β and TNFα, with upregulation of STAT6 and CD163.

DISCUSSION: These results demonstrate that cLIUS reprograms macrophages exposed to injury-relevant Fnfs from a catabolic M1-like state toward an M2-like reparative phenotype. This shift was supported by suppression of inflammatory gene networks and activation of wound-healing and anti-inflammatory signaling pathways.

SIGNIFICANCE: This study identifies cLIUS as a novel non-pharmacologic therapeutic to modulate macrophage polarization in the context of Fnfs-induced inflammation, relevant to early PTOA pathogenesis. The findings have translational implications for cartilage and synovium diseases, injury healing, mechanobiology, and therapeutic development in orthopedics.

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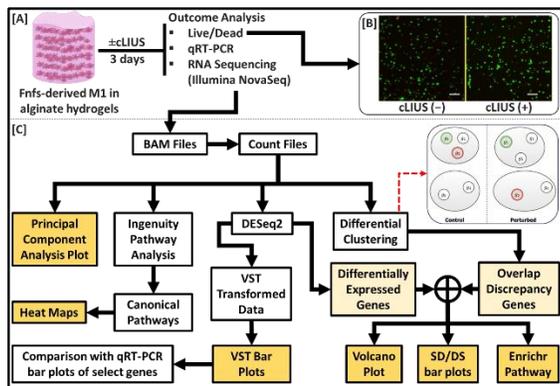


Figure 1: (A) Experimental design; (B) Viability; (C) Workflow

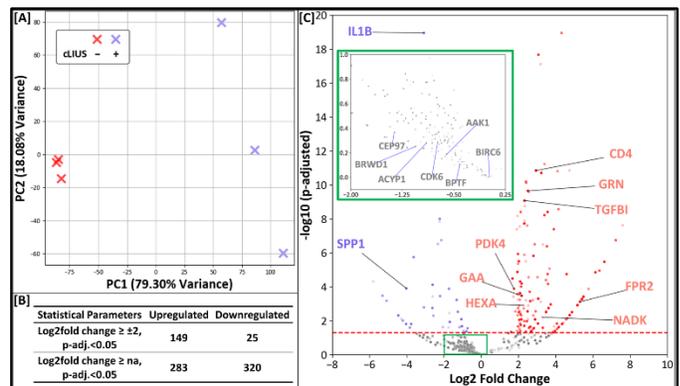


Figure 2: (A) PCA plot: cLIUS vs Ctrl; (B) DEGs statistics; (C) Volcano plot

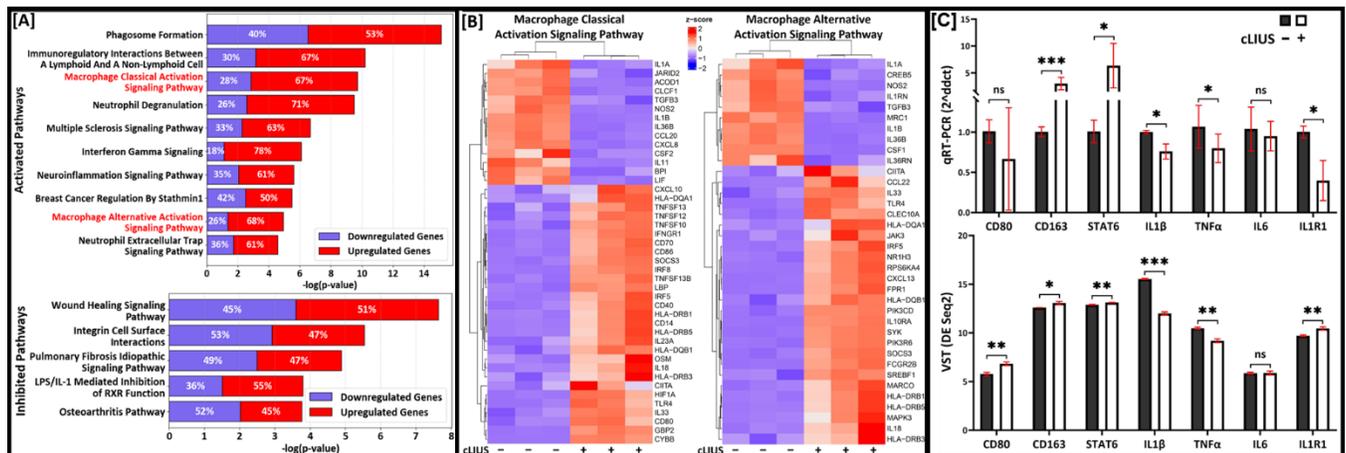


Figure 3: (A) Top activated and inhibited IPA pathways; (B) Heatmaps of classical & alternative activation signaling pathways; (C) qRT-PCR vs VST