**Unraveling the Transcriptome Profile of Pulsed Electromagnetic Field Stimulation in Bone Regeneration Using a Bioreactor-Based Investigation Platform**

Farah DAOU – *University of Eastern Piedmont*

## INTRODUCTION

Pulsed electromagnetic field (PEMF) stimulation has gained increased attention in bone and cartilage repair, and is widely applied in clinical practice. Several signaling pathways related to its osteogenic, chondrogenic, and anti-inflammatory effects were determined, but most of the pathways are still unknown. Rigorous testing is required to gain deeper insights into the associated signaling pathways and to optimize and standardize clinical protocols in terms of waveform, amplitude, frequency, and exposure dose. This work aimed to uncover the signaling pathways elicited by PEMF using a novel *in vitro* investigation platform that exposes 3D trabecular bone-like tissues to bone-like mechanical stimulation and PEMF stimulation.

## EXPERIMENTAL METHODS

*Bioreactor.* An automated perfusion bioreactor for culturing in parallel up to 3 bone-like tissues under tunable direct perfusion (0.006-24 mL/min) with supplemental PEMF stimulation (1.5 mT, 75 Hz), was adopted (Fig. 1A). *Scaffolds*. Polylactic acid (PLA) scaffolds (total porosity = 60%, average pore size = 600 µm) were 3D printed to resemble trabecular bone microarchitecture. *Biological evaluations.* Scaffolds were seeded with human mesenchymal stem cells (hMSCs) and exposed to perfusion (0.3 mL/min) with and without PEMF stimulation (4 h/day) for 21 days in basal or osteogenic medium (Fig. 1B). Static cultures served as control. RNA sequencing (RNA-Seq) and real-time qPCR were conducted to detect the signaling pathways elicited by PEMF.

## RESULTS AND DISCUSSION

The PEMF effects on cells are evident in the absence of biochemical stimuli. RNA-Seq revealed that PEMF stimulation in basal medium targets the four phases of bone healing: inflammatory, fibrovascular, bone formation, and bone remodeling phases, even in the absence of a pathological state (Figure 1C).

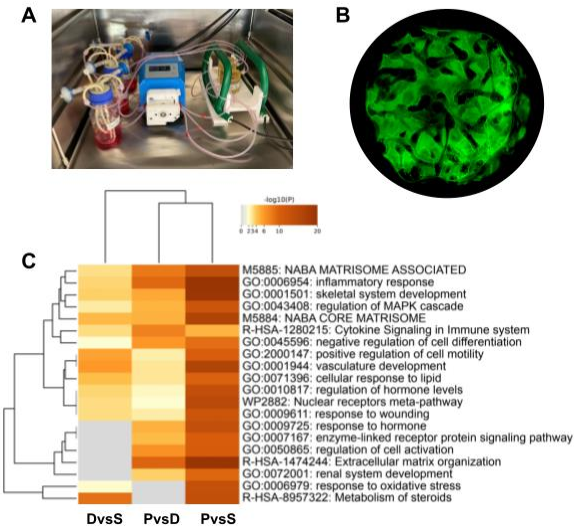


***Figure 1**: (A) In vitro investigation platform; (B) Live/dead assay after 21 d of direct perfusion in basal medium; (C) GO enrichment analysis. S: static; D: direct perfusion; P: Direct perfusion with PEMF stimulation.*

## CONCLUSION

The proposed *in vitro* investigation platform allowed for uncovering the signaling pathways activated by PEMF and represents a powerful tool for bone biology research.