# 3D PRINTED CONDUCTIVE SCAFFOLDS COMBINED WITH ELECTRICAL STIMULI FOR IMPROVED BONE TISSUE ENGINEERING STRATEGIES

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## Introduction

The high incidence of non-union fractures caused by trauma or disease in an aging population has increased the clinical demand for tissue-engineered bone. Electrical stimulation (ES) has been shown to improve bone repair in several research/clinical studies [1, 2]. Nevertheless, the biological mechanisms by which ES enhances bone formation are still not fully understood and its application in bone tissue engineering (BTE) strategies is currently underexplored. Additive manufacturing (AM) methods (e.g. Fused Deposition Modeling-FDM) have been widely employed in BTE due to their ability to produce scaffolds with a high control over their architecture, porosity and mechanical properties in a scalable and reproducible manner [3]. Therefore, in this study, we combined AM technologies with electrically conductive scaffolds and ES to enhance the osteogenic differentiation of human bone marrow-derived mesenchymal stem/stromal cells (hBMSCs) envisaging improved BTE strategies.

## Methods

Electro-bioreactor devices were fabricated by FDM using medical-grade electrode materials (stainless steel and Ti6Al4V) to apply ES to 2D cell cultures and 3D cell-seeded scaffolds. Finite Element Analysis (FEA) was employed to estimate the magnitude and distribution of electrical fields within the ES devices and along the conductive scaffolds. Five different ES protocols were applied to 2D hBMSC cultures for 14 days to select the best performance one in terms of cell proliferation and osteogenic differentiation. Then, the selected protocol was used in two different scaffold-based BTE strategies: i) different conductive scaffolds (conductive poly lactic acid (cPLA), conductive thermoplastic polyurethane (cTPU) and titanium) were seeded with hBMSCs and cultured for 21 days under osteogenic medium conditions with and without ES and their biological performance was evaluated in comparison to non-conductive scaffolds; ii) several PEDOT:PSS-based coating solutions were screened to obtain PEDOT:PSS-coated polycaprolactone (PCL) scaffolds, in which hBMSCs were cultured for 21 days under osteogenic induction with and without ES. The biological performance of the scaffolds (with and without ES) was evaluated in terms of cell viability/proliferation as well as typical bone-specific extracellular matrix (ECM) production and gene expression (immunofluorecence / RT-qPCR analysis).

## Results

All 3D porous conductive scaffolds tested were able to support hBMSC viability, migration and proliferation. The fabricated 3D PEDOT:PSS-coated scaffolds (Figure 1) presented a high electroconductivity (11.3-20.1 S/cm), which values were stable for 21 days in saline solution. When cultured under exposure to ES, the best performance coating condition scaffold (PEDOT:PSS/Gelatin-PCL) was shown to improve significantly the osteogenic differentiation of hBMSCs *in vitro*, in particular, their cell-secreted calcium deposition (mineralization), bone-specific ECM protein expression and osteogenic marker genes upregulation (in comparison to all other experimental groups).

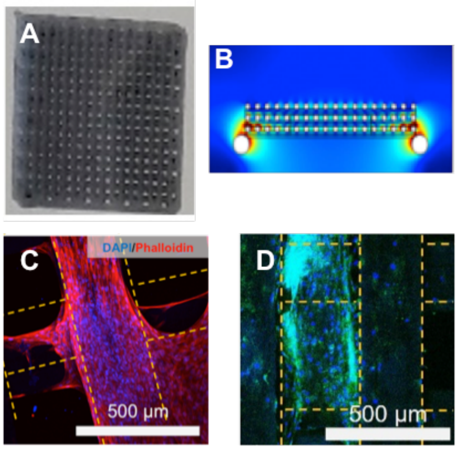


*Figure 1: A) Gross image of 3D PEDOT:PSS/Gelatin-coated scaffold; B) FEA model predicting the electrical fields distribution in 3D PEDOT:PSS/Gelatin scaffold; C) hBMSCs morphology observed through DAPI/Phalloidin staining after 7 days of culture in 3D PEDOT:PSS/Gelatin scaffolds; D) Collagen Type I expression (green) by differentiating hBMSCs after 21 days of culture in 3D PEDOT:PSS/Gelatin scaffolds under osteogenic induction media and ES exposure.*

## Discussion

Our results demonstrated clearly a synergistic effect of 3D AM-based scaffold conductivity and ES on the improvement of the osteogenic differentiation of MSCs, highlighting the potential of their combined use for novel personalized BTE clinical applications.

## References

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29th Congress of the European Society of Biomechanics, June 30 – July 3, 2024, Edinburgh, Scotland