3D-Printed Conductive Polymeric Scaffolds with DC Electrical Stimulation for Enhanced Bone Regeneration

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Statement of Purpose: Various methods have been explored to treat bone defects caused by genetic disorders, injury, or disease. However, there is still great need to develop alternative approaches to regenerate damaged bone tissue. Healthy native bone has the ability to generate endogenous electrical charges that stimulate the reparative process through voltage-controlled calcium channels [1]. It has been observed that with critical-sized bone defects, these signals are compromised; however, as bone begins to heal, the bioelectric potential at the site of injury returns back to normal, suggesting that bone regeneration can be supported by repairing the bioelectrical microenvironment [2]. 3D printing has emerged as a key tool for creating rapid, solvent-free synthetic bone scaffolds for tissue engineering with defined geometries and controllable porous architectures. However, the polymers typically used for this technique lack biological activity and tend to serve primarily as void fillers. Thus, the combination of 3D-printed scaffolds made of conductive polymers and externally-applied direct current (DC) electrical stimulation (ES) could be an advantageous approach for bone repair by exploiting the known physiochemical properties of natural bone. This study aimed to demonstrate the potential of conductive polymer-based composites as a means for enhanced bone formation under stimulation.

Methods: An ES platform was fabricated by fitting two L-shaped platinum electrodes 10mm apart into the lid of a 24-well plate, with spacing allowing for electrode placement into each well. The electrodes were soldered into a parallel circuit that could be connected to a power source via alligator clips. A commercially-available extrusion-based 3D printer (Monoprice) was used to print circular, thin-film scaffolds consisting of either polylactide (PLA) or a conductive PLA (CPLA) composite with resistivity of 30 Ω-cm along 3D printed layers (Protoplant). Both the PLA and CPLA scaffolds, were soaked overnight in a dopamine hydrochloride solution. After a preliminary study to optimize an ES protocol for treating cells, MC3T3-E1, murine preosteoblasts cells, were used to assess cellular attachment, proliferation, and differentiation on the scaffolds with and without ES applied. Cells were seeded on scaffolds at 38,000 cells/well in 24-well Ultra-Low Attachment plates (Corning) and grown to confluence over 5 days in proliferation media, before being cultured in osteogenic differentiation media for a total of 24 days in culture. Cells cultured on the scaffolds were subjected to DC ES (100mV/mm continuously for 5 min daily) at least four times per week for a total of 14 days while cultured in osteogenic media. Relative cell viability was assessed, in addition to measurement of early and late-stage osteogenic markers for bone formation using ELISAs. Samples for quantitative assays were run in triplicate (n=3). Statistical analysis was performed using two-way ANOVA followed by Tukey post-tests for multiple comparisons. The significance level was set to $p < 0.05$ (GraphPad Prism).

Results: Treatment of the scaffolds with the dopamine hydrochloride solution resulted in more hydrophilic scaffold surfaces, as demonstrated by a decrease in the contact angles measured following coating. Both PLA and CPLA scaffolds allowed for cellular attachment, proliferation, and differentiation over 24 days with no cytotoxic effects observed (data not shown). The custom-made DC ES chamber showed promise as a means to electrically stimulate bone precursor cells *in vitro* using the described stimulation protocol. Specifically, when applied to cells cultured on conductive scaffolds, ES treatment resulted in a significant increase in the expression of osteocalcin, a protein indicative of osteoblast maturation, after 14 days of culture (**Figure 1A**). In addition, staining of cells using Xylenol Orange demonstrated the presence more mineralized calcium nodules for cells undergoing stimulation, when compared to cells on CPLA without stimulation (**Figure 1B**).

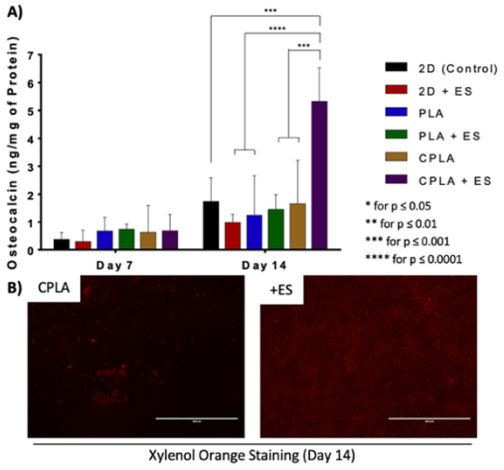

Figure 1. A) Osteocalcin expression over 14 days of osteogenic differentiation. **B)** Xylenol Orange staining of mineralized calcium nodules present within the cultures of cells seeded on conductive scaffolds without/with (right/left) ES at Day 14.

Conclusion: Pre-osteoblasts were successfully able to attach, proliferate and differentiate on conductive scaffolds without a cytotoxic response, when compared to 2D controls. In conjunction with ES, conductive scaffolds resulted in enhanced mineralization potential, as well as increased osteogenic protein expression, compared to both 2D controls and non-conductive scaffolding materials. Overall, this study demonstrates the potential for conductive scaffolding materials combined with ES to treat bone defects through restoration of the bioelectric microenvironment. Future work will include further exploration of this modality for bone regeneration.

References: [1] M Griffin et al., Eplasty 11, e34 (2011).
[2] RA Gittens et al., J Dent Res 90 (12), 1389 (2011).