Fabrication of A Biodegradable and Electroactive Foundation for Bone Regeneration

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INTRODUCTION: Bone fractures, particularly those that are large or poorly heal, continue to be a growing concern within the orthopaedic community given their susceptibility to lead to delayed unions or nonunions. As a potential method to enhance bone healing, we propose fabricating scaffolds using melt electrowriting, an advanced 3D printing technique, to serve as a foundation for bone regeneration. These scaffolds are printed using a biodegradable, biocompatible, and electroconductive polymer made via a mixture of polycaprolactone (PCL) and PEDOT. In order to support proper osteogenesis, the composite scaffolds must be able to maintain structural stability and mimic the mechanical properties of the surrounding native bone tissue while promoting cell attachment and proliferation. Previously, we have successfully been able to produce and print the PEDOT-PCL composite scaffolds. The purpose of our work now is to evaluate the cytotoxicity, biocompatibility, and mechanical properties of these fabrications to determine if they are viable substrates for recapitulating the bone environment. We propose that this novel biodegradable and electroactive scaffold will serve as a viable substrate for improved bone regeneration in nonunion fractures.

METHODS: PEDOT nanoparticles were synthesized by oxidative polymerization of the 3, 4-ethylenedioxythiophene monomer (EDOT) in the presence of ferric chloride. PCL was mixed with PEDOT and melted in a microwave to create mixtures of 0%, 0.5%, 1%, and 1.5% PEDOT concentration. The composite polymers were then loaded into an Axo-A3 3D bioprinter (Axolotl Bio) with melt-electrowriting capabilities and printed using an electrical field of 5 kV. Subsequently, the scaffolds with the various concentrations of PEDOT were assessed using a Texture Analyzer for further evaluation of their mechanical properties. Next, scaffolds were loaded with osteoblasts and transferred to an empty well plate to account for the number of cells that were able to successfully adhere to the scaffolds. AlamarBlue assay was used to elucidate scaffold cytotoxicity and osteoblast proliferation over the course of 1, 3, and 7 days. Concurrently, cell-scaffold adhesion was further analyzed via DAPI staining over a period of 1, 3, and 5 days. Statistical analyses were performed with Prism 7 (GraphPad Software Inc). Statistical differences between groups were determined by a Student's t-test. Significance was accepted at $p < 0.05$.

RESULTS: In regard to mechanical evaluation, the average peak force withstood and the average tensile strength of scaffolds significantly increased as the concentration of PEDOT increased compared to PCL alone ($p<0.05$). Each PEDOT-PCL scaffold group exhibited at least a 2-fold increase in these parameters with the 1.5% PEDOT-PCL scaffolds having the greatest peak force and tensile strength out of all groups (Figure 1). No difference was seen in the elastic modulus between groups. The 1.5% PEDOT-PCL group also demonstrated the highest difference in reduction of AlamarBlue compared to all other scaffold groups after 7 days. Scaffolds fabricated with 1% PEDOT-PCL and PCL alone were found to have a similar reduction in AlamarBlue and 0.5% PEDOT-PCL scaffolds resulted in the lowest AlamarBlue conversion (Figure 2).

DISCUSSION: Our findings indicate that increasing the concentration of PEDOT within the polymer mixture results in scaffolds that display the greatest tensile strength and can endure the most force applied by the Texture Analyzer. Although a similar relationship was not seen with osteoblast metabolic activity (indicated by AlamarBlue reduction), interestingly, of all scaffold groups, the 1.5% PEDOT-PCL composite demonstrated the greatest tensile strength and peak force endured while also allowing for the highest proliferation and metabolic activity of its respective osteoblastic cells.

SIGNIFICANCE/CLINICAL RELEVANCE: Our research demonstrates the possibility of constructing a scaffold foundation that not only demonstrates a mechanical integrity capable of enduring higher forces but is also conducive for the growth of human osteoblastic cells. Ultimately, our future research will be to implement these promising scaffolds within *in vivo* bone defects to promote bone regeneration in poorly healing fractures and fractures at risk for nonunion.

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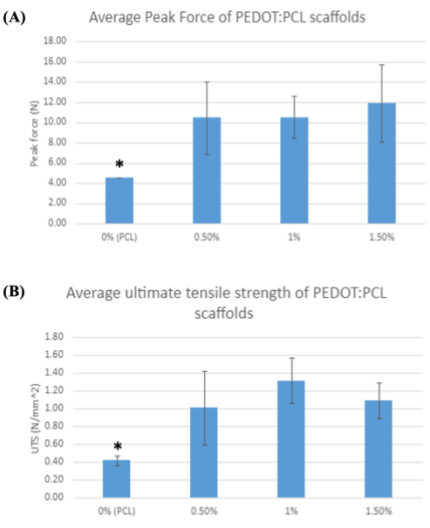

Figure 1 - Mechanical Testing.
(A) Average peak force endured by each scaffold group sorted by PEDOT:PCL concentration. **(B)** Average ultimate tensile strength of each scaffold group sorted by PEDOT:PCL concentration. **(*)** indicates significance of PEDOT:PCL findings compared to PCL alone.

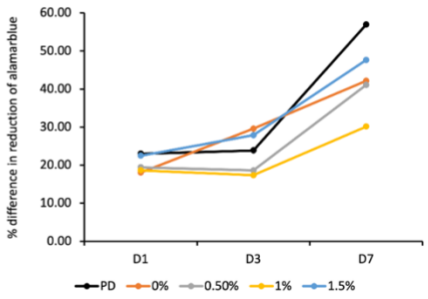

Figure 2 – Osteoblast Proliferation.
Percent difference in reduction of alamarBlue recorded at days 1 (D1), 3 (D3), and 7 (D7). Line plots color-coded by PEDOT:PCL concentration. PD indicates osteoblast control group without scaffold. Reduction in alamarBlue demonstrates osteoblast proliferation and metabolic activity.