Using Electric Fields to Introduce Pores in Bacterial Cellulose Scaffolds for Tissue Engineering

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Statement of Purpose: Tissue engineering, specifically through the use of biomaterials, provides a promising alternative to harvested tissue via the incorporation of cells with bio-engineered scaffolds that mimic the extracellular matrix. However, the major challenge in tissue engineering is the inability to create functional three dimensional tissue structures in vitro that mimic the extracellular matrix and allow for proper gas and nutrient exchange amongst cells. To increase cell viability, pores can be integrated into scaffolds to increase cell migration and proliferation. Multiple techniques such as sintering, salt leeching, gas foaming, and electrospinning have been used to create micro structured architectures within scaffolds. Although these techniques allow for fairly uniform pore distribution, they do not allow for proper pore interconnectivity, which fails to induce microvasculature formation. This work explores using electric fields to control 3D networks of cellulose produced by the bacterial strain *Gluconacetobacter xylinus*. Bacterial cellulose is an ideal scaffolding material due to its biocompatibility, mechanical integrity, and its stability under a wide range of conditions. Our approach uses varying electric field pulses to incorporate conduits into the cellulose. We hypothesized that using electric fields to control the bacteria at specific locations and particular times, we could introduce conduits in the overall scaffold by preventing cellulose biosynthesis locally**.**

Methods: Through mathematical modeling and experimental techniques, electrical effects were of *Gluconacetobacter xylinus* were determined. To determine bacteria cell death threshold experimentally, bacteria cells were taken from suspension and electrical pulses were delivered using a BTX® Square Wave Electroporation System with a safety stand (Harvard Apparatus, Holliston, MA) using a varying set of pulse parameters. Untreated bacteria and bacteria soaked in CAVICIDE™, a medical grade disinfectant, were used as positive and negative controls, respectively. The samples were then reseeded on agar plates to monitor cell growth after the application of electrical pulses. To create a localized conduit, electrical pulses were delivered through the BTX® system with a varying set of pulse parameters to active cultures of *Gluconacetobacter xylinus.* A FE analysis was used to calculate the electric field distribution experienced on the cellulose scaffold and correlated to the experimental results to determine the electric field threshold needed to form a conduit.

Results: The bacteria cell death threshold was determined. As the electric field is increased, there was a noticeable decline in the percent growth of bacteria compared to the positive control. Here, we show that electric fields can be used to effectively control *Gluconacetobacter xylinus*. The field contour maps shown in the FE model predict the lesion area created on the surface of the pellicle, which can be compared to the macroscopic and FESEM images of the BC. Samples treated at varying pulse parameters successfully created conduits in the cellulose scaffold of varying sizes.

Conclusions: Tissue engineering, through the use of scaffolds, holds great promise for treating some of the most devastating diseases of our time. The major challenge thus far is attributed to the manufacturing of stable and functional 3D tissue structures, with appropriate porosity and microvasculature, to allow long-term viability of complex organs such as bone. Our approach to incorporate conduits into the cellulose scaffold is through the use of electric fields. In this proof of concept study, we successfully showed that electric fields can be employed to control the architecture of a cellulose scaffold. Electric fields are therefore, an innovative biofabrication technique to create pores in BC scaffolds; it allows for high degree of control over the bulk lattice structure and thus better constructs with native tissue architecture. Our next step is to automate and refine this fabrication process in order to develop custom-made scaffolds with various porosity densities to satisfy the requirements for orthopedic implants and other applications.

References:

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