Electromagnetic Stimulation of a Nanoparticle-Tissue Scaffold

Daniel N. Grant[1], Richard White[2], David Grant[3], Sheila Grant[3]
School of Medicine[1], Department of Orthopaedic Surgery[2], Department of Bioengineering[3]
University of Missouri, Columbia MO

Statement of Purpose: The goal was to investigate the effects of extremely low frequency electromagnetic fields (ELF-EMF) in promoting cellularity in nanoparticle-bioscaffolds which could be used to treat orthopaedic injuries. The utilization of ELF-EMFs was an attempt to replicate the "current of injury" to expedite the wound healing process. Studies have shown that ELF-EMFs will improve healing rates [1]. Studies have also shown that the utilization of gold nanoparticles (AuNPs) in tissue scaffolds will enhanced biocompatibility and reduce inflammation [2]. In theory, local magnetic fields and electrical currents created by the AuNPs might enhance cell proliferation. It was hypothesized that AuNP-bioscaffolds will allow for enhanced cellular in-growth and proliferation from cellular interactions with the nanoparticles and from the local fields generated around the nanoparticles by ELF-EMFs.

Methods: Decellularized porcine diaphragm tendon was utilized as the tissue scaffold. Porcine diaphragm tendons were procured following euthanization of swine at the University of Missouri School of Medicine. The porcine diaphragm was decellularized using a treatment consisting of tri(n-butyl) phosphate. The scaffolds were assigned as crosslinked, crosslinked with 1x AuNPs, or crosslinked with 4x AuNPs. Crosslinking was performed using a solution consisting of acetone and PBS, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, and N-Hydroxysuccinimide. Functionalized 100 nm AuNPs were conjugated via zero-length crosslinker to the tissue. After conjugation, the bioscaffolds were sterilized using a solution comprised of 1M sodium chloride and 0.1% (v/v) peracetic acid for 24 hours.

A pair of Helmholtz coils separated by a distance equal to their radii was utilized to generate a uniform magnetic field over an area 14cm in diameter. A polyvinyl chloride (PVC) cell culture chamber was fabricated to incubate the cells in the magnetic field. Gas ports allowed for a steady flow of 5% CO_2 from a commercial-grade cell culture incubator. Heated water from a commercial water bath system was pumped through the PVC cell culture chamber to maintain a temperature of 37°C. A 12 Gauss, 60 Hz ELF-EMF was utilized. The bioscaffolds were exposed to the ELF-EMF for 2 hours each day for 3, 7, and 10 days. Modulated differential scanning calorimetry (mDSC) was utilized to determine the structure stability. A Quant-iT^{TM} PicoGreen double stranded DNA quantification assay was performed to measure the cellular proliferation using L-929 mouse fibroblast cells.

Results: mDSC: The denaturation temperatures were utilized to assess the stability of the bioscaffolds. The mDSC results revealed that the denaturation temperatures were not statistically significant amongst the 3 sample groups: cross-linked, 1x AuNP, 4x AuNP, with the average denaturation temperature of 74.99°C, 76.75°C, and 78.45°C respectively.

In Vitro: Figure 1 shows the change in DNA concentration over time without error bars. For the unstimulated 1x AuNP and 4x AuNP scaffolds, there were significantly greater DNA concentrations from day 3 to day 10 ($p<0.05$ and $p<0.01$ respectively). For the stimulated crosslinked, 1x AuNP, and 4x AuNP scaffolds, there were significant greater DNA concentrations at day 10 compared to day 3 ($p<0.01$ for all). For the cross-linked samples at 7 and 10 days, the DNA concentration was significantly greater for the simulated group compared to the unstimulated group. There were no significant differences between the simulated and unstimulated groups at 7 or 10 days for the 1x AuNP or 4x AuNP samples. However, on average, the 1xAuNP-stimulated samples had higher cellularity than the 1xAuNP-nonstimulated samples at each time point.

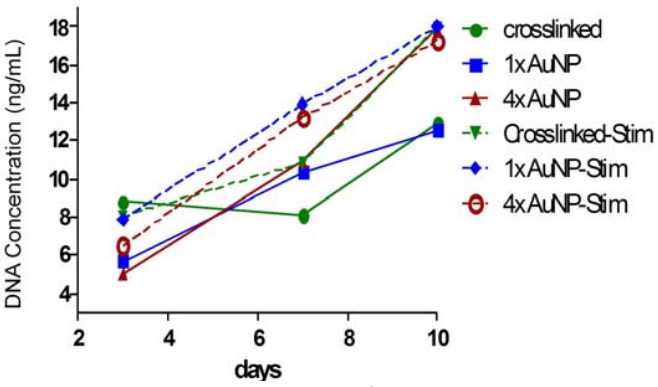

Figure 1. DNA concentration of the stimulated and unstimulated scaffolds.

Conclusions: The results of the mDSC suggest that the concentration of AuNPs on the scaffold does not adversely affect the stability of the bioscaffolds.

The Quanti-iT^{TM} PicoGreen dsDNA assay revealed a strong correlation between ELF-EMF stimulation and cell proliferation. These findings highlight the potential positive effects ELF-EMF stimulation. The presence of AuNP on the unstimulated scaffolds showed enhanced cellularity as compared to the crosslinked only samples. This indicated beneficial effects of the presence of gold nanoparticles conjugated to the scaffolds over just the crosslinked scaffolds. Stimulated versus unstimulated scaffolds conjugated with AuNPs did not result in significantly different cell proliferations, but average cellularity was higher in the AuNP stimulated samples as compared to AuNP unstimulated. The results of the study provided some interesting scientific information on ELF-EMF and cellularity effects. It also highlights the need for further investigations regarding ELF-EMF effects and nanoparticle-bioscaffolds.

References: 1. **(**Callaghan MJ. Plast Reconstr Surg 2008; 121:130-141 2008.); 2. (Deeken CR. J Biomed Mater Res B. 2011; 96:351-359.)