Poly(lactic-co-glycolic acid) (PLGA) Composites with Magnesium Wires Enhanced Networking of Primary Neurons

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Statement of Purpose: The current gold standard in peripheral nerve repair is the nerve autograft. However, this procedure requires additional surgery to harvest graft material, and has the risk of neuroma formation. (Nectow A. Tissue Engr B. 2012;18:40-50.) Tubular conduits, known as nerve guidance conduits (NGCs), offer an alternative solution. Several NGC devices manufactured from biodegradable synthetic and natural polymers have been approved by the FDA, but the performance of most of these conduits has not yet approached the level of autografts. (Kehoe S. Injury. 2012;43:553-72.) Strategies to improve performance include providing cues for directional outgrowth of neurons and incorporating bioactive molecules to promote neuroinduction and neuroconduction. In this work, we incorporated magnesium wires into a 50:50 poly(lactic-co-glycolic) acid (PLGA) scaffold to provide both directional and biological cues in a fully bioresorbable material. Magnesium (Mg) is important for many physiological processes and Mg ions are neuroprotective agents used clinically to prevent excitatory nerve damage in conditions ranging from eclampsia in pregnant women to ischemic stroke (Perlman, J. Clin Therapeutics. 2006;28;1353-65). Additionally, a recent *in vivo* study in mice reported that systemic delivery of Mg salts after sciatic nerve crush injury resulted in enhanced regeneration of the injured nerve (Pan, H. Mg Rsrch. 2011;24;54-70). These findings motivate us to investigate the incorporation of Mg as a bioactive component in PLGA scaffolds.

Methods: Thin films of PLGA were prepared using a solvent casting method in a Teflon mold. Briefly, 50:50 PLGA (PolySciTech, West Layfeyette, IN) was dissolved in chloroform with heat and sonication to create a 5% w/v solution. A base layer of PLGA was cast, then Mg wires with a diameter of 125 µm (Goodfellow, Coraopolis PA) were degreased and cleaned sequentially in acetone and ethanol with sonication, and placed in the PLGA scaffold at equal distance. Each 1x1 cm scaffold contained 10 wires per cm. A second layer of PLGA was cast on top of Mg wires, embedding wires between two layers of PLGA. Scaffolds were dried in a chemical hood for 24 hours and under vacuum for an additional 48 hours to ensure complete removal of solvent. The resulting scaffolds were sputter coated and imaged using a scanning electron microscope (Nova NanoSEM 450) to characterize the main features of the scaffolds. The 1x1cm scaffolds were sterilized for 1 hour in 100% ethanol, and coated with poly-D-lysine (PDL) and laminin (Sigma-Aldrich, USA) before neural cell culture. Hippocampi were isolated from pups at embryonic day 18, digested with papain and DNAse (Life Technologies, USA), triturated, and filtered with a cell strainer (BD Falcon, USA). The resulting cell suspension was seeded in 24 well plates containing either the prepared scaffolds or PDL and laminin coated glass cover slip control. Cultures were incubated for 8 days under standard cell culture conditions (5% CO_2 with 9% O_2) and then fixed with 4% paraformaldehyde. Scaffolds and cover slips were incubated overnight with antibodies for microtubule-associated protein-2(MAP2) (Sigma-Aldrich), which binds to neuron dendrites. The next day samples were mounted on glass slides and nuclei stained with Vectashield + DAPI (Vector Laboratories, Burlingame CA) for fluorescence imaging using Nikon Eclipse Ti.

Results: SEM images showed full, uniform coverage of wires within the PLGA. Fluorescence images taken at 8 days showed distinct morphologies between neurons grown on the PLGA-Mg composite scaffolds and those grown on glass. Neurons on the scaffolds formed denser networks with more interconnected and branching dendrites. In addition, dendrites extending along and over the wires were observed.

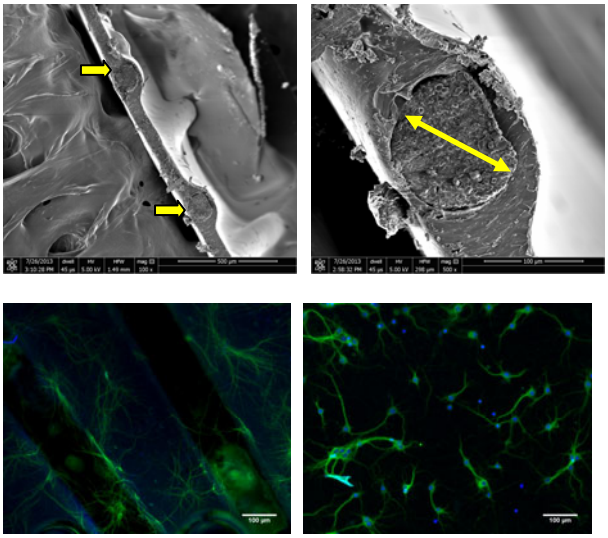

Figure 1: (a,b) SEM images of (a) PLGA-Mg scaffold cross-sections (scale = 500µm) and (b) Magnified image of Mg wire cross-section (scale = 100µm). yellow arrows indicate Mg (c,d) Fluorescence images of hippocampal neurons grown for 8 days (10X magnification, scale = 100µm) on (c) PLGA-Mg scaffold and (d) glass cover slips.

Conclusions: Mouse hippocampal neurons grown on PLGA-Mg composite scaffolds showed distinctive differences in morphology and network density compared to control cultures grown under the same conditions on glass cover slides. In addition, the observed interactions between neuronal dendritic projections and Mg wires may indicate that the topology of these scaffolds provides directional cues for neuronal growth. These results suggest that our composite scaffolds are promising as a potential candidate material for NGCs. Further studies are still needed to quantify these results and look for specific interactions between neurons and PLGA-Mg composite scaffolds.