Interaction of Bone Marrow-Derived Macrophages with Highly Aligned Electrospun Fiber Scaffolds for Neural Engineering Applications

Han Bing Wang[1], Elizabeth J. Hager[2], Sherri L. Wiseman[2], Ryan J. Gilbert[1]
[1]Biomedical Engineering, Michigan Technological University, Houghton, MI, USA; [2]Biological Sciences, Michigan Technological University, Houghton, MI, USA.

1. Statement of purpose: Constructing highly aligned, electrospun fibers is important in directing neurite extension on biomaterials [1]. In the current study, to better understand the interaction of macrophages with our aligned, electrospun fiber scaffolds, bone marrow-derived macrophages were cultured on aligned fibers and planar films (control) made from poly-L-lactic acid (PLLA, NatureWorks, Minnetonka, MN). After macrophage maturation, lipopolysaccharide (LPS) was added to the culture media. The morphology of the cells was assessed and the inflammatory response was studied by measuring nitrite production. It is important to understand this interaction since adhesion of macrophages to fiber scaffolds may be detrimental to regenerating axons within the spinal cord. Previously, it was shown that activated macrophages interact negatively with sparred neurons following spinal cord injury [2].

2. Methods: ***2.1 Fibers fabrication and characterization:*** Highly aligned PLLA electrospun fibers were fabricated using an electrospinning technique [1]. Before electrospinning, a layer of PLLA film was cast onto coverslips to secure fiber position and alignment following electrospinning. The working conditions for electrospinning were the following: working voltage: 14 kV; working distance: 11 cm; flow rate: 2 ml/hr; using a 22 gauge sharp-tip needle. The fiber samples were coated with 5 nm gold, and the images were taken using a field emission scanning electron microscope.

2.2 Bone marrow-derived macrophage isolation and maturation: Bone marrow cells were obtained by flushing the center of femurs and tibiae with macrophage media. Cells were filtered and cultured on PLLA films (as a control) and highly aligned PLLA fibers at a density of 1×10^6 cells per coverslip. L-929 (Invitrogen, Carlsbad, CA) conditioned media was changed every 2 days until day 6. At day 7, the media was replaced with media without L-929.

2.3 NO release assay and analysis of cell morphology: At day 8, the media (without L-929) was replenished with media containing 500 ng/mL of LPS (Sigma, St. Louis, MO). At 0h, and after 24h and 48h, the culture supernatant was collected for NO measurement using the Griess assay (Invitrogen, Carlsbad, CA) [2]. Samples were stained using calcein-AM (Sigma, St. Louis, MO) for 0h, 24h and 48h.

2.4 Statistical analysis: A one-way ANOVA (JMP IN 8.01) was run to determine statistical difference between groups.

3. Results: ***3.1 Morphology of macrophages on polymer film and aligned fiber samples:*** Cells were plated on film or aligned fibers for 7 days. Fig 1-A shows a SEM image of the fibers. On aligned fibers, the cells were stretched along the fibers (Fig 1-B). On films, the mature cells exhibited the typical morphology of macrophages (Fig 1-C).

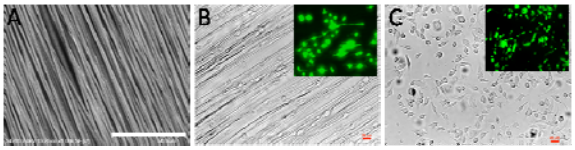

Figure 1: (A) SEM image of aligned fibers. (B) Brightfield and calcein-stained images show macrophages stretched on aligned fibers. (C) Brightfield image and calcein-stained images show macrophages cultured on planar films presenting a typical morphology. Scale bar =50 μm in (A), Scale bar=20 μm in (B)/(C).

3.2 Nitrite production: LPS induced nitric oxide (NO) production at 24h and 48h was measured using the Griess assay kit. As shown in Fig. 2, the amount of nitrite produced by macrophage activated by LPS for 24h and 48h was significantly increased ($p<0.05$). The peak of NO production for macrophage on the films appeared at 24h (17.3±1.3 μM), while for cells on aligned fibers, the NO peak was at 48h (20.5±0.5μM). Therefore, the aligned fibers may delay the maximum activation of stimulated macrophages.

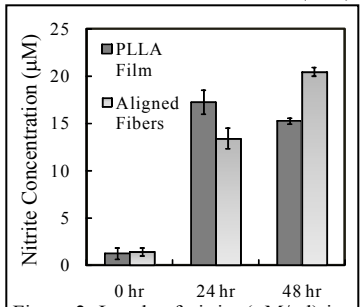

Figure 2: Levels of nitrite (μM/ml) in culture supernatants from mice bone marrow-derived macrophage stimulated with LPS (500ng/ml) for 0h, 24h and 48h.

4. Conclusions: As the activation of macrophages on aligned fiber scaffolds remain unclear, the interaction of bone marrow-derived macrophages with aligned fibers was investigated. Macrophage cells were cultured on PLLA films and aligned, electrospun PLLA fibers. On aligned fibers, cells were stretched along the fibers. NO release results show that fibers may delay the activation of macrophages after stimulation. More studies (cytokine release, varying fiber parameters) analyzing the interaction between macrophages and aligned fibers will be pursued in the future.

5. References: 1. Wang, H.B., et al., J Neural Eng, 2009. **6**(1):016001.
2. Horn, K.P., et al., J Neurosci, 2008. 28(38): 9330-41.
3. Sacco, R.E., et al., Comp Immunol Microbiol Infect Dis, 2006. 29(1):1-11.