Fabrication and Characterization of Immunomodulating Electrospun Fibrous Mesh

Isabel D. Wallgren[1], Ming Ying Yeoh[1], Mentor: Hannah R. Childs[1], Fereshteh Zandkarimi[2], Helen H. Lu[1]
[1]Department of Biomedical Engineering, [2]Mass Spectrometry Core Facility, Columbia University, New York, NY

Introduction: Scarring is associated with extra-cellular matrix dysregulation[1] and myofibroblast activation and persistence.[2] Nuclear factor κB (NF-κB) is correlated with impaired healing of rat rotator cuff tendons,[3,4] human tendon scarring,[5] and fibrotic diseases.[6] NF-κB inhibition reduces myofibroblast activation *in vitro*[6] and promotes tendon healing *in vivo*.[4] 2-Amino-6-[2-(cyclopropylmethoxy)-6-hydroxyphenyl]-4-(4-piperidinyl)-3 pyridinecarbonitrile (ACHP) suppresses the NF-κB inflammatory subunit IKKβ.[4] The objective of this study was to incorporate ACHP into collagen-based fibers to locally target NF-κB *in vitro* to reduce scarring and promote tendon and ligament regeneration.

Methods: A polymer melt of 40% gelatin, 50% acetic acid, and (+/-) ACHP was electrospun per Mosher et al.[7] Meshes were crosslinked with glutaraldehyde under vacuum. 6 mm discs were biopsy punched, sterilized under UV light, and cultured in F/S DMEM media at 37°C for 24 hours (n=5). Release media was centrifuged, and supernatant collected, dehydrated, and resuspended for liquid chromatography mass spectrometry (LC-MS). *As fabricated* meshes (n=4) were homogenized and similarly prepared for LC-MS. Scanning electron microscopy (SEM, 5 kV, Zeiss Sigma VP)[7] images (5000X, n=50-60 fibers, 10-12 images) were analyzed using Image J to determine mean fiber diameter. Fiber alignment was measured using MatFiber.[8] ACHP – biopolymer interactions were assessed with Fourier transform infrared spectroscopy in attenuated total reflectance mode (FTIR-ATR, n=3, Spectrum 100, Perkin Elmer). Loading efficiency and percent ACHP released was determined via LC-MS.

Results: The ACHP loading efficiency was 72.7%, of which 70.9% of ACHP was released into media within 24 hours. Fiber diameter decreased with ACHP incorporation while fibers were unaligned for both groups.

Conclusions: We developed and characterized mechanically stable and physiologically relevant collagenous scaffolds demonstrating localized ACHP release. Future research is required to achieve optimal, controlled release for sustained ACHP delivery. Incorporation of ACHP into biopolymer scaffolds offers a potential method to target the NF-κB inflammatory pathway.

References:

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