Cationic nanofibers as anti-inflammatory scaffolds for chronic wound healing.

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Statement of Purpose: Dead and dying cells release extracellular nucleic acids (NA) that can activate the inflammatory pathways of immune cells. Over-activation of these pathways is a factor contributing to sustained inflammation related to chronic wounds, as well as chronic inflammation stemming from autoimmune diseases like systemic lupus erythematosus, rheumatoid arthritis and inflammatory bowel disease. The Sullenger lab has previously demonstrated that certain soluble, cationic polymers can scavenge extracellular nucleic acids and inhibit RNA-and DNA-mediated activation of Toll-like receptors (TLRs) and inflammation[1]. We hypothesize that the incorporation of cationic polymers onto insoluble nanofibers would enable the scavenging of pro-inflammatory species directly from blood, reducing cytotoxicity related to unwanted internalization of the polymers. Herein, we report preliminary, *in vitro* data to support that electrospun nanofibers grafted with cationic polymers can absorb agonists of TLR 3,7, 9 directly from serum and prevent the production of NF-κβ, an immune system activating transcription factor while also demonstrating low cytotoxicity.

Methods: Unaligned fibers were fabricated by electrospinning a solution of 6% or 10% (w/v) Poly (styrene-co-maleic anhydride) in 1:1:1 tetrahydrofuran, dimethylformamide, and acetone at 15V. Nanofibers were collected on a fly-wheel rotating at 30 revolutions per minute. Cationic fibers were made by soaking the electrospun PSMA fibers in 0.1M 1.8kDa branched poly(ethylenimine) (bPEI) for 48hrs, then washed with DI water, and sterilized with ethanol for 20 min. Nanofibers were imaged and characterized using SEM. Cell viability studies were performed in mouse fibroblast cells (STO) and a B lymphocyte cell line (Ramos Blue™, Invivogen). Cell viability was determined by direct contact of the cells with the fibers. Cell-activation and specificity studies were performed with Ramos Blue™ cells by submerging fibers in serum free media with NA and non-NA based TLR agonists and subsequently treating the cells with the fiber-exposed medium. Resulting NF-κβ levels were measured using QUANTI-Blue™ (Invivogen), a secreted embryonic alkaline phosphatase (SEAP) detection medium.

Results: SEM shows that the fibers are random and in the nanometer range of 270-380nm and 800-900nm for 6% and 10%, respectively. Cell viability studies with STO and Ramos blue cells show minimal toxicity of the fibers upon direct contact with the cells. The minimal toxicity of the cationic polymers, 6%/10%+bPEI in **Fig. 1B** is presumably due to the increased basicity from bPEI. Cationic nanofibers effectively eliminated the immune stimulating response of NA based agonists CpG (TLR 9), and poly (I:C) (TLR 3) **Fig. 1C**. Results show that unmodified PSMA fibers have no inhibitory effects, demonstrating that the fiber activity is not due to a physical interaction with the fibers. The cationic fibers (6%/10%+bPEI) reduced the Ramos Blue™ NF-κβ response to the baseline of un-stimulated cells. Specificity of the 6% cationic fibers to scavenge only NA based agonists was further tested using non-NA, cationic TLR agonists (R848, pam3CSK4) as controls. **Fig. 1D** shows that the cationic fibers selectively inhibit the activity of the NA based agonists, CpG and Poly(I:C).

Conclusions: Electrospun PSMA fibers modified with bPEI can inhibit the activation of Toll-like receptors (TLRs) pro-inflammatory nucleic acids. These cationic nanofibers show specificity for negatively charged agonists and demonstrate promise for developing novel dressings and treatments for inflammation in chronic wound healing in which a sustained immune response prevents completion of wound healing, leaving wounds open and exposed to further infection. A cationic fiber bandage has potential to eliminate immune agonists and promote wound healing. We are currently determining an appropriate animal model to test the utility of these nanofibers for chronic wound healing and inflammation.

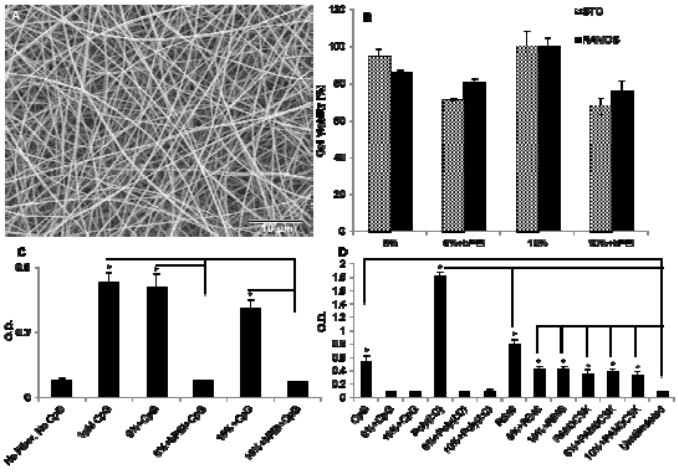

Figure 1: (A) SEM image of 6% PSMA electrospun fibers modified with bPEI. (B) Cell viability of STO and Ramos Blue cells after 4 hrs of incubation with fibers. (C) NF-κβ response of Ramos Blue cells after CpG stimulation in the presence of unmodified fibers (6%+CpG, 10%+CpG) or cationic fibers (6%+bPEI+CpG, 10%+bPEI+CpG). (D) NF-κβ response of Ramos Blue cells after stimulation with negatively charged agonists (CpG, Poly(I:C)) or positively charged agonists (R848, PAM3CSK); treatment with cationic fiber is stipulated by the 6% or 10% in front of the agonist descriptor.

References:

1) Lee J, Sohn JW, Zhang Y, Leong KW, Pisetsky D, Sullenger BA. Proc Natl Acad Sci U S A. 2011;108(34):14055-60.

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