Antimicrobial-Loaded Microhydrogels Self-Assembled on Polymeric Nanofiber Scaffolds

Qichen Wang, Radoslaw Junka, Xiaojun Yu, Matthew Libera
Stevens Institute of Technology, Hoboken, NJ, United States

Statement of Purpose: Electrospun polymeric nanfibers have been used as fibrous matrices to mimic the structure of the extracellular matrix (ECM) in biological tissue. However, in nanofiber mattes used as tissue scaffolds, desirable tissue cells and undesirable bacteria can compete for the colonization of fiber surfaces. Because of their small size, however, bacteria can penetrate into a fibrous matte more easily than larger tissue cells and colonize the matte there with little or no competition from tissue cells. To address this problem, we are exploring the functionalization of nanofiber mattes using self-assembled poly(ethylene glycol) [PEG] -based hydrogel particles. PEG diacrylate (PEGDA) was copolymerized with acrylic acid (AA) by surfactant-free emulsion polymerization to form gel particles with diameters ranging from ~50 – 500 nm. These particles were then electrostatically deposited on PCL-chitosan nanofibers. Antifouling PEG gels can by themselves reduce bacteria adhesion. In addition, however, each gel particle can be loaded with antimicrobial peptide to further inhibit bacterial colonization. This method to functionalize fiber mattes occurs in a simple, two-step, non-line-of-sight deposition process that leaves much of the fiber surface unmodified and still adhesive to desirable tissue cells.

Methods: The fabrication of PCL-chitosan nanofibers used solutions of 8% (w/v) PCL and 0.8% (w/v) chitosan in hexafluoroisopropanol (HFIP, Oakwood products). These two solutions were mixed to form a solution of 1% (vol/vol) chitosan to PCL and stirred overnight to form a uniform clear solution. The electrospinning conditions were set at 10 µl/min, 10 cm distance and an operating voltage of 12 kV. The fibers were collected on a grounded aluminum foil. We worked with nanofiber mattes ~50 µm thick. An emulsion polymerization process was used to synthesize PEG hydrogel particles. 200 µl of PEG 575 diacrylate, 20 µl acrylic acid and 10 µl Darocur 1173 photoinitiator were dissolved in 1 ml of dichloromethane and then dispersed in 10 ml of DI water by sonication for 20 min. The resulting emulsion was exposed to UV light to drive free-radical polymerization during 15 min of additional sonication. After purification, the gel particles were electrostatically deposited onto PCL-chitosan nanofibers by soaking the fiber into pH 7.4 particles suspension. A solution of L5 polycationic peptide at pH 7.4 was dropped onto gel-modified fiber mattes, left there for 2 hrs, and then washed away using pH 7.4 PBS buffer. *S. epidermidis (S. epi)* was innoculated for 1 hr under gentle shaking and at 37 ^{o}C on bare fibers, gel-modified fiber, and peptide-loaded gel-modified fiber. The fiber matte specimens were then incubated for 4 more hours at 37 ^{o}C, and bacterial response was studied by confocal fluorescence optical microscopy.

Results: Zeta potential and dynamic light scattering measurements confirm that the as-synthesized gel particles have a pH-dependent charge and size because of the copolymerized acid groups. At pH 7.4, these gel particles are negatively charged. Because of the positive charge associated with the chitosan, the gels can infiltrate the entire nanofiber matte and electrostatically bind to the fiber surfaces. Confocal imaging (fig. 1) confirms that the gel particles infiltrate the matte (red) and the FITC-labeled peptide (green) infiltrates the individual gel particles. The bacteria-response study (fig. 2) shows the peptide-loaded gel particles dramatically reduce the extent of bacterial S. epi colonization.

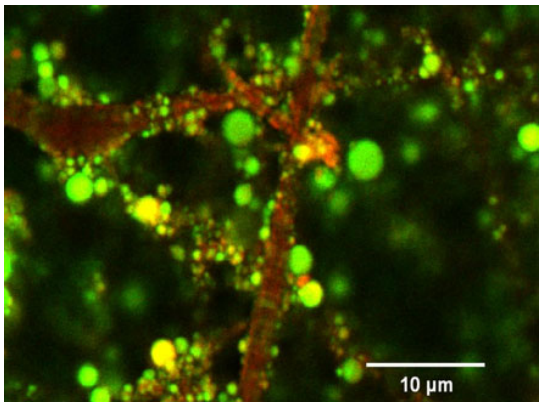

Fig. 1 - PCL-chitosan nanofibers (red) modified by peptide-loaded gel particles (green).

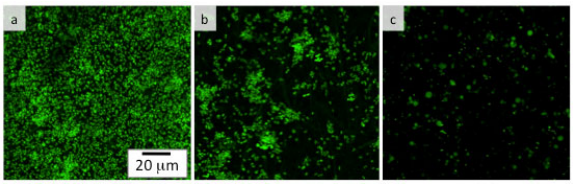

Fig. 2 - After 4 hrs incubation, *S. epi* covers almost the entire surface of unmodified nanofiber matte (a). S. epi colonization can be significantly reduced by incorporating antifouling gel particles (b), and further reduced using L5-modified gel particles (c).

Conclusions: Nanofiber mattes used for tissue-engineering scaffolds present a significant amount of internal surface structure, which is susceptible to bacterial colonization with little or no competition from tissue cells or from the immune response. Self assembly enables electrostatically charged microhydrogel particles to be deposited throughout the matte structure. Subsequent exposure to polycationic peptides enables these gel particles to be electrostatically loaded, thus creating internal reservoirs of antimicrobials.