Surface Grafting of Chitosan-Coated Electrospun Fibers to Promote Cell Adhesion

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Statement of Purpose: Electrospinning of polyesters (e.g., polycaprolactone (PCL)) is an attractive approach for fabricating meshes with mechanical properties suitable for orthopaedic tissue engineering applications. However, the resultant fused-fiber meshes are biologically inert, necessitating surface grafting of bioactive factors, such as the peptide sequence RGD to stimulate cell adhesion. Introduction of primary amine groups for bioconjugation using diamines (i.e., aminolysis) is transient. Therefore, we adapted the process of Zhang[1] to co-axially electrospin chitosan-coated PCL micro-fibers, where the primary amines of chitosan are available for subsequent grafting.

Methods: Co-axial electrospinning was used to fabricate fibers containing a PCL core and a chitosan/polyethylene oxide (PEO) shell. The PCL was prepared as a 9 wt% solution in trifluoroethanol, and a 1.5 wt% chitosan/0.5 wt% PEO solution was prepared in an aqueous 1.5% acetic acid, 2% Tween 80, and 10% DMSO solution. Co-axial electrospinning was performed using a +15 kV potential, 18 cm throw distance, and a flow rate of 0.5 ml/h for both phases, and collected on a slowly rotating mandrel (~20 rpm) covered with aluminum foil. To visualize the shell phase, the chitosan solution was doped with 5 µg/ml of FITC. To confirm the availability of primary amines on chitosan for bioconjugation, carboxy-rhodamine was attached to electrospun fibers via carbodiimide chemistry. Briefly, 0.49 mg/ml carboxy-rhodamine, 1.44 mg/ml EDC and 0.17 mg/ml NHS in a solution of 0.1 M 2-(N-morpholino) ethanesulfonic acid buffer (pH 5.5) were incubated with the fibers overnight, rinsed, and imaged. RGDC was conjugated to fibers via sulfo-SMCC. Briefly, fibers were incubated with 200 µl (4 mg/ml) sulfo-SMCC linker for 1 h at room temperature, followed by overnight incubation with 200 µl of RGDC (125 µg/mL) overnight at 4 °C.

Results: Co-axial electrospinning resulted in meshes consisting of 1.3 µm diameter micro-fibers with a PCL core and chitosan/PEO sheath. Mechanical testing indicated a tensile modulus of co-axial micro-fiber meshes of 10.1 MPa (measured wet), that agreed well with the 5.7 MPa modulus of wet PCL micro-fiber meshes. Imaging of fluorescently labeled co-axial fibers revealed a FITC signal of the chitosan phase that persisted after overnight soaking (Figure 1). Next, to demonstrate that primary amines are available for conjugation, carboxy-rhodamine was grafted to fibers using EDC/NHS chemistry. In particular, fluorescence was significantly attenuated by the omission of EDC indicating covalent attachment of rhodamine (Figure 2). Lastly, the adhesive peptide RGDC was grafted to chitosan via the thiol group of cysteine using sulfo-SMCC. Mesenchymal stem cells – seeded onto the fiber meshes – attached and spread more quickly on the RGD-tethered microfibers as compared to fibers without RGDC (Figure 3).

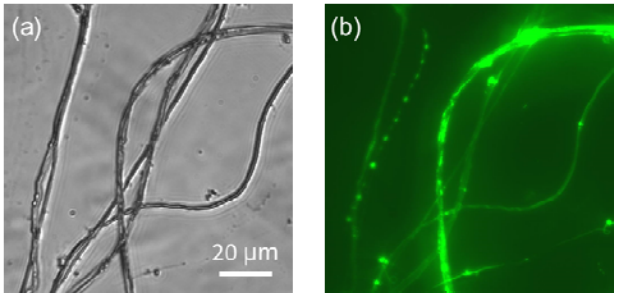

Figure 1. a) Phase contrast and b) fluorescent image of micro-fibers prepared with a PCL core and a FITC-labeled chitosan/PEO sheath after overnight soaking in deionized water.

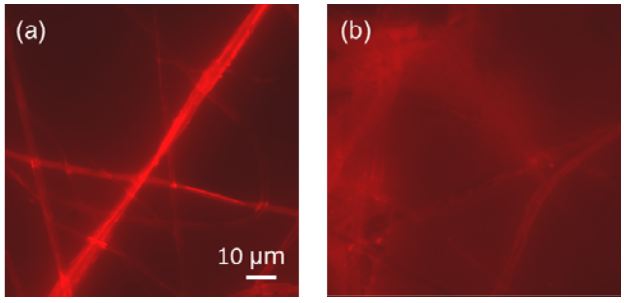

Figure 2. Fluorescence images of carboxy-rhodamine conjugation of micro-fibers a) with EDC and b) without EDC. The former corresponds to specific binding and the latter, non-specific adsorption of dye.

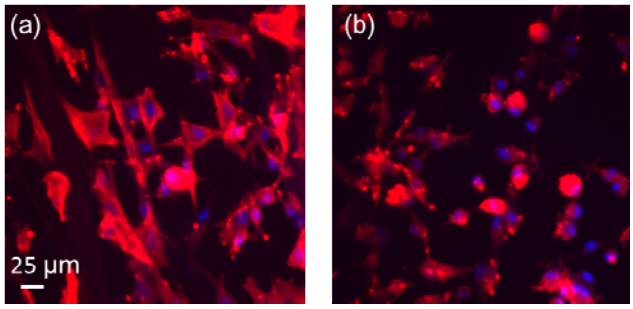

Figure 3. Fluorescence images of cells on a) RGD conjugated micro-fibers and b) RGD adsorbed to micro-fibers. Red corresponds to F-actin, and blue to nuclei.

Conclusions: The results suggest that co-axial electrospinning can produce meshes comprised of micro-fibers with PCL cores and chitosan sheaths. Further, the chitosan sheath phase appears to be stable in water, and the primary amines in the chitosan are available for subsequent bioconjugation. Bio-conjugation of RGDC to microfibers improves initial cell adhesion and spreading.

References:

(1) Zhang YZ. Biomacromolecules. 2007;9:136-41.