Modular Tissue Engineering: Chitosan-GAG Fibers Formed From an Ionic Complex

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Modular tissue engineering is a promising approach to current regenerative medicine issues. Modules containing cells can be used as a building block for a larger structure, eventually correlating to an engineered tissue. In general, any module must be able to induce an environment favorable for cell growth while retaining mechanical properties suitable for a specific application. Chitosan-polyanion ionic complex membranes can be used to encapsulate and culture a variety of cell types. Hollow fibers of these materials also have the potential to be used in a variety of tissue engineering applications in vitro and in vivo. The chitosan provides structure, is biocompatible, and can be modified for specific mechanical properties. Glycosaminoglycans (GAGs) provide an environment favorable for tissue engineering applications because of their wide range of biological activity that can be tailored to specific tissue engineered systems. In this study we investigated and developed procedures for continuous generation of chitosan-polyanion hollow fibers with cells or other materials incorporated during fiber formation.

A polyanion solution, consisting of 1.5 wt% medium-viscosity carboxymethylcellulose and 4.0 wt% chondroitin 4-sulfate dissolved in sorbitol-HEPES buffer, was layered over a solution of 0.6 wt% chitosan and 3.8 wt% sorbitol in 0.06 % acetic acid was introduced horizontally into a solution of chitosan in sorbitol via a needle and syringe pump setup. Fibers were drawn from the ionic complex membrane formed at the chitosan-polyanion solution interface. Fibers were pulled coaxially away from the needle and through the chitosan solution using tweezers to initiate extraction. These fibers were collected by pulling out of the chitosan solution and were either rolled onto a cylindrical rod or pulled vertically until failure. The ability to encapsulate materials within the fibers was examined by suspending these materials in the polyanion solution prior to drawing. Materials investigated included suspensions of fixed hepatocytes, mesenchymal stem cells, and microcarriers.

The fibers drawn had diameters ranging from 0.2-1.0 mm after being extracted by hand from a liquid-liquid interface of 7.74mm^2. These fibers achieved a maximum length of ~10cm when lifted vertically. If rolled onto a cylindrical rod, the fiber continued to form until a solution was depleted. The polyanion solution was introduced into the chitosan solution at a rate of 1.0 $^{ml}/_{min}$ Fibers generated were observed to be hollow by the evidence of both microcarriers and cells enveloped within the complex fiber. Fibers with cell suspensions placed in culture fractured and disintegrated within 24 hours and is thus the frontier of our ongoing work.

These fibers are promising candidates for modular tissue engineering applications. These fibers can also be generated with materials of interest inside the fiber walls. Fibers generated with cells and matrix components can be assembled into 2D or 3D structures for engineered tissue assembly and analysis. Our ongoing work entails making these fibers strong enough to withstand static culture conditions with viable cells and matrix components inside. Once a suitable fiber formulation has been identified, characterizing cell growth, organization, and

function are important qualities to study for future progress. A system for continuous, controlled rate fiber extraction is also being implemented to determine a correlation between fiber properties and extraction parameters.