Preparation of Electrospun Chitosan-PEO Fibers

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***Abstract*—Chitosan and PEO were dissolved in acetic acid in order to be electrospun for tissue engineering scaffold purposes.**

I. Introduction

1.1 Tissue Engineering

Tissue engineering is an interdisciplinary field merging chemistry, biology, and biomaterial and mechanical engineering principles to create viable design replacements for failing tissues and organs. In order to overcome the immunosuppressive response of a host and promote intraceullular homeostatic conditions, there are certain biological and physical requirements for tissue substrates. The tissue substrate must be biocompatible – both structurally and biochemically, bioresorbable, elastomeric and non-toxic during its degradation. The biopolymer(s) must serve as a temporary scaffold for cells to attach, organize, multiply, and participate in respiration and digestion. The three dimensional structure of the tissue scaffold should elicit a functional response thus encouraging cellular differentiation and neo-tissue formation. Therefore, the scaffold should be porous and exhibit a high surface area to volume ratio to guide the cellular development and migration.

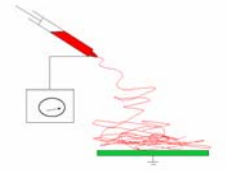
Electrospun polymer materials show promise as temporary matrix substitutes because they are characterized as nonwoven, three-dimensional, interconnective, nanofibrillar networks, which are suitable for cellular attachment and differentiation.

1.2 Electrospinning

Electrospinning is the method of obtaining fibers on a submicron to nanometer scale by applying a voltage to a polymeric solution or melt. To perform a simple electrospinning experiment, one would need a syringe to store the solution, a needle, a power supply, and a grounded collector target. At a given critical voltage, the surface tension forces in the solution are overcome and a Taylor cone is formed at the tip of the syringe. Once the solution viscosity is overcome, the apex of the Taylor cone stretches across the electric field towards a region of lower potential, forming a continuous jet of fluid. Experimentally, it was determined that the nature of the jet instability region, or whipping jet, contributes to the formation of submicron fibers due to the stretching and acceleration of the polymer jet in the instability region. The whipping of the jet is caused by the interaction between the external electric field and the surface charge of the jet. While the jet whips and bends, the solvent quickly evaporates from the jetted fluid, and the solidified nonwoven polymer fibers are collected on the grounded target.

$$V_c^2 = 4\frac{H^2}{L^2}\left(\ln\frac{2L}{R}-\frac{3}{2}\right)(0.117\pi\gamma R) \quad (1)$$

Vc = critical voltage
H = distance between the capillary tip and the ground
L = capillary length
R = capillary radius
γ = surface tension of the liquid



There are many solution and process parameters that govern the morphology and structure of the collected fibers. Polymer solution parameters include viscosity, surface tension, dielectric constant of the solvent, solution conductivity, concentration and M_w. Electrospinning process parameters include voltage, feed rate, tip-to-collector distance, diameter of needle and temperature of the solution. Other factors include humidity and vapor pressure of the surrounding environment.

1.3 Polymers

$$d = \left[\gamma\varepsilon\frac{Q^2}{I^2}\frac{2}{\pi(2\ln\chi^{-3})}\right]^{1/3} \quad (2)$$

Where d = fiber diameter;
γ = surface tension;
ε = dielectric constant;
Q= flow rate;
I = current;
X = jet length/nozzle diameter

In this study, various types of chitosan and polyethylene oxide (PEO) solutions were electrospun in order to create scaffold networks that will conjointly be tested with a number of cell cultures to promote cellular viability, cell signaling and communication, and cell mobility for tissue engineering purposes. PEO was added to improve the structural ability and charge carrying capacity of the polymer solution. Chitosan and PEO are natural polymers, thus making them nontoxic, less likely to elicit an immune response, and they also express high degrees of hydrophilicity – the ability to retain moisture. Chitosan is the N-deacetylated derivative of chitin, the most abundant natural amino polysaccharide on Earth and is found in crustaceans, insects, and fungi. Chitosan is a popular choice for tissue engineers because it contains an amine group, making it easy to be chemically modified with the addition of growth factors or morphogenic proteins, and it can bind easily to divalent metal ions. The addition of chitosan to any scaffold also increases the mechanical integrity of the scaffold. Chitosan is a polymeric amine, basic in nature, and its chemical structure is poly-(1-4)-2-amino-2-deoxy-β-D glucose. Chitosan is important in wound healing and tissue repair, and it also shows excellent cell adhesive properties.

II. Materials and Methods

90% pharmaceutical grade deaceytlated chitosan (high degrees of acetylation promote better substrates for tissue engineering and improve fiber strength) was mixed with PEO

in 1:1, 2:1, 3:1 and 5:1 ratios and dissolved in 2% (wt/v) acetic acid. PEO with a molecular viscosity weight of 600kDa was purchased from Sigma-Aldrich (USA). A 0-20kV power supply was used from Gamma High Voltage (Model KC Kilovolts, USA) and a syringe pump was used to control the feed rate (New Era Pump System Inc.). Positive voltage was directed to the needle and negative voltage was directed to the collector plate to complete the circuit. The circular collector plate with a surface area of 7mm^2 was made out of aluminum foil. Temperature and relative humidity readings were taken for each test.

2.1 Solution preparation and characterization

Ratios of chitosan (90% deaceytlated) to PEO (600kDa), 1:1, 2:1, 3:1, and 5:1 were dissolved in 0.5 M / 2% (wt/v) acetic acid to a final volume of 20 mL. Solutions were mixed overnight at room temperature. pH values of each homogenous solution were measured with an UltraBasic Benchtop pH Meter (Denver Instrument), viscosity readings were taken with a Brookfield Digital Viscometer, and bright field spectra waveforms were recorded.

2.2 Electrospinning

Each solution was placed in a 10-mL syringe with a hypodermic needle, ranging from 16-20 gauge, depending on the viscosity of the solution. The power supply was set up for a positive voltage of 17 kV. The syringe pump was set at 0.1mL/hour. The collector plate was set 10 cm away from the tip of the needle, and was held upright by a clamp and ringstand. At the time of the experiments, relative humidity and temperature values ranged from 18-26% RH and 24-28°C.

2.3 SEM Imaging

Fiber samples 5mm*5mm were sputtercoated for 35 seconds with platinum and images were taken with the Amray 1830 Scanning Electron Microscope at 20kV and a working distance of 8mm. Fiber diameter and pore size distributions in the fiber samples were noted.

III. RESULTS

Sample	Viscosity (cP)
2% AA	7446.2
PEO in AA	19633.5
Chitosan in AA	199144
1:1 Chitosan to PEO (.4g)	3193.1
1:1 Chitosan to PEO (.6g)	5516.9
1:1 Chitosan to PEO (.8g)	5031.03
2:1 Chitosan to PEO	3435.7
5:1 Chitosan to PEO	11666

Figure 1: Viscosity Measurements

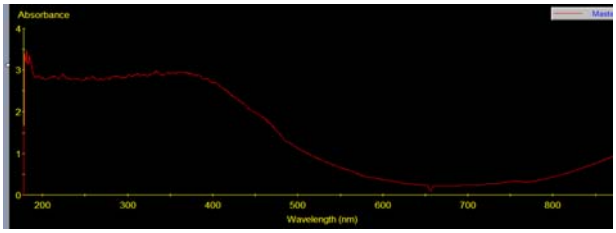


Figure 2: Spectra for 1:1 Chitosan-PEO

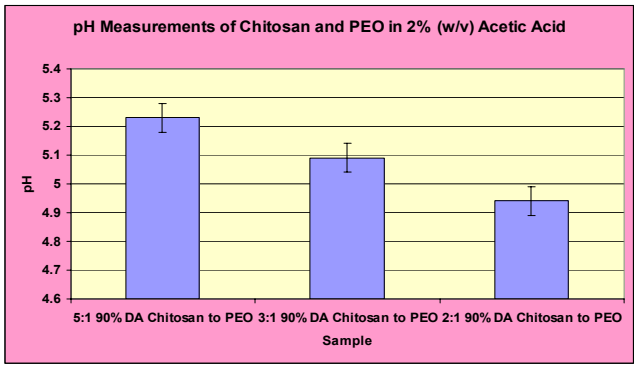


Figure 3a: pH Measurements of Chitosan-PEO

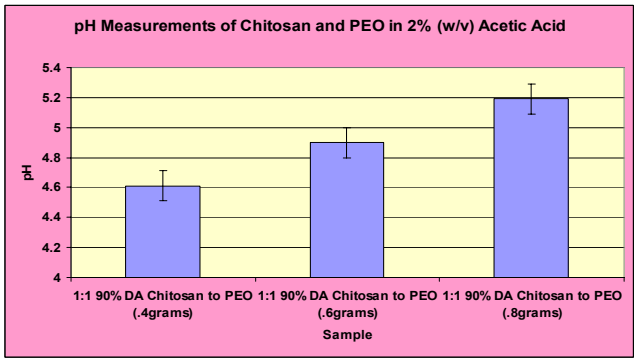


Figure 3b: pH Measurements of Chitosan-PEO

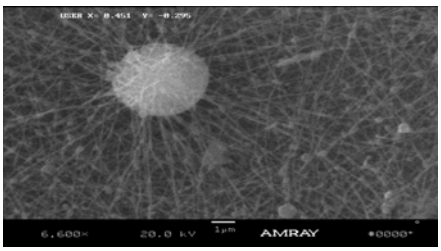


Figure 4*: 2:1 90% DA Chitosan to PEO

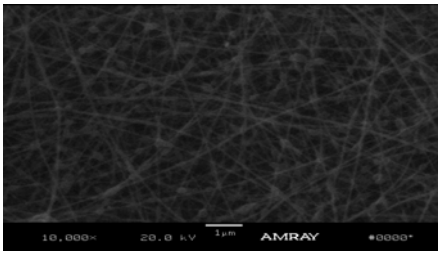


Figure 5*: 1:1 (.6g) 90% DA Chitosan to PEO

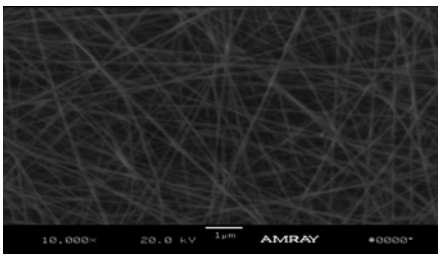


Figure 6*: 1:1 (.4g) 90% DA Chitosan to PEO