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A novel nanofiber scaffold by electrospinning and its utility in microvascular tissue engineering

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Abstract

Cellulose acetate (CA) thin, porous membranes were produced by electrospinning precursor polymer solutions in acetone at room temperature. During this process, CA nanofibers were produced when a high electric field of 12 kV was applied to the precursor solution. The diameters of fibers obtained varied from 100 nm to 1.2 μm while the average diameter was approximately 500 nm. The electrospinning parameters used to control the morphology of the fibers and their membranes are flow rate, the distance between the syringe needle that ejects fluid and the collector, and the voltage applied. These membranes were used as scaffolds for microvascular cells growth. The structure of the membranes that were produced mimic the topography and porosity of extracellular matrix (ECM) in two key ways. The fiber diameter mimics extracellular protein fiber diameter, thus enabling cellular attachment and facilitating cellular migration. The porosity mimics that of extracellular matrix such that microvascular capillary tube formation is enhanced. The non-woven fiber mats were examined by means of electron microscopy and the nanofibers were seen to be oriented randomly. The issue of strengthening the CA scaffold is currently studied by adding ceramic nano-structured component (carbon nanotubes) in the polymer membranes.

Keywords: electrospinning, cellulose acetate, microvascular, tissue engineering

1. Introduction

In 1934, Formhals [1] patented a novel process for producing polymer fibers by using electrostatic force, which is called electrospinning. In this process, two electrodes are attached to the capillary of a syringe which contains the polymer solution and the collector respectively. Under the applied electric field, a polymer jet is formed and deposited on the collector, usually metal screen [2]. In this experiment, dry deposition conditions were used, only solutes can be finally deposited on the collector due to the evaporation of the solvent during the ejection process. By means of controlling the voltage of the electric field, the flow rate of the polymer solution, the distance

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between the capillary and the collector and the concentration of the polymer solution, the diameters of nanofibers can be tailored easily. Table 1 summaries the process and solution parameters tested [3].

Table 1 Summary of the process and solution parameters [3].

Parameters of the electrospinning process	Flow rate of the polymer solution
	Voltage
	Distance between the syringe and the collector
Parameters of polymer solutions	Concentration
	Viscosity
	Surface tension

In the present work, we produced a Cellulose Acetate (CA) based nanofiber scaffold by electrospinning. By adding a second component, carbon nanotubes, CT, into the CA solution, CA-CT composite membranes were fabricated and used as scaffolding for endothelial cell growth. Endothelial cells form the inner lining of all blood vessels; their directed growth is essential for wound healing, and tissue regeneration. To facilitate vascular endothelial cell growth on these membranes, process parameters were optimized to mimic the structure of the natural extracellular matrix, such as collagen, which naturally forms a network of 0.5-10μm diameter fibers [4]. Endothelial cell will migrate along the collagen fibers in the early phase of new microvessel growth [5]. The fabricated CA-CT membranes specifically mimic the topography and porosity of natural extracellular matrix (ECM).

2. Experimental Methods

2.1 Electrospun materials

Cellulose Acetate with a number average molecular weight of 29,000 g/mol was purchased from Fluka (Fluka Chemie GmbH CH-9471 Buchs). Acetone, ACS, 99.5+% (Assay) was obtained from Alfa Aesar, MA, USA. Those two were used for preparing the electrospinnable polymer solutions. As-prepared single walled carbon nanotube (AP-SWNT) was obtained from Carbon Solutions Inc., CA, USA, and used as a secondary ceramic nanoparticles in the polymer membranes for strength.

Solutions of CA and acetone with compositions ranging from 7.5 to 20.0% w/v were prepared simply by mixing them together at room temperature (ca. 22-25℃). The polymer dissolved completely in acetone usually after 2.5 h without stirring. Cellulose Acetate-Carbon Nanotube (CA-CT) solution was prepared by directly adding the nanotube to 15.0 % w/v CA acetone solution. In order to obtain homogeneous solution, the CA-CT solution was made under stirring. Precipitation of nanotubes occurs after 5 min. Therefore, the electrospinning process of CA-CT solution was done within 5 min after the completion of solution preparation.

Different compositions of CA solutions were electrospun under a voltage as high as 12kV with the distance between the syringe and the collector being ca. 15 cm. The flow rate was controlled at 100 μ l/min. CA-CT solution was electrospun under the same condition.

2.2 Endothelial cell culture

Human umbilical vein endothelial cells were obtained as first passage cultures. Cells were incubated at 37° in a 5% CO_2 humidified atmosphere. The cell culture media was composed of McCoy's 5A base media with 20% calf serum, 100μg/ml heparin, 50μg/ml endothelial cell growth supplement, 2mM L-glutamine, without antibiotics (Sigma Chemical Co.) [6]. Coverslips and membranes were sterilized by uv light exposure.

Three types of electrospun materials were tested for their effect on cellular viability: CA alone, CA+0.25%CNT, CA+0.5%CNT. Cells were trypsin digested to transfer onto $1cm^2$ area membrane at a dilution of 1:1 or 1:2. On days 3-5 post seeding, cellular viability was assessed in two ways; cells were either loosely attached and could be washed off by rinsing the material, or tightly attached and remained bound to the material after rinsing. To determine the viability of loosely attached endothelial cells, we used a trypan blue (Sigma Chemical Co.) assay on the effluent from the material wash. To determine the viability of the tightly attached endothelial cells, we used calcein/ethidium (Molecular Probes, Inc) fluorescence directly on the material; this latter method was also used on the control cells on coverslips.

Light Microscopy. Living endothelial cell in culture were viewed with fluorescence and phase contrast microscopy (Nikon E800) using 4x, 20x or 40x objectives. Trypan blue assay was assessed at 4x using a hemocytometer and appropriate counting statistics. Calcein/ethidium assay was assessed using fluorescence (Chroma filter sets) at 40x. Color images were taken with an Evolution QEi camera system (Media Cybernetics, and analyzed using Qimaging software.

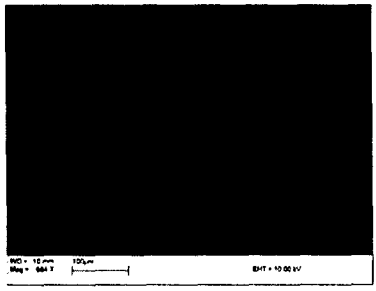
Material porosity – hydraulic conductivity. The materials used with cells were evaluated for functional porosity by testing hydraulic conductivity of double thickness materials. A sheet of material was folded and gently placed between two gaskets that were sealed into a Teflon tubing line; the cross-sectional area of material open for water flow was $3.1x10^{-2}$ cm^2. Water was pumped through the material by syringe pump (0.01-1ml/min) and the balance pressure was determined. The slope of the balance pressure as a function of volume flow was used to estimate porosity, using the equation: flow = (hydraulic conductivity x surface area) x pressure.

3. Results and Discussion

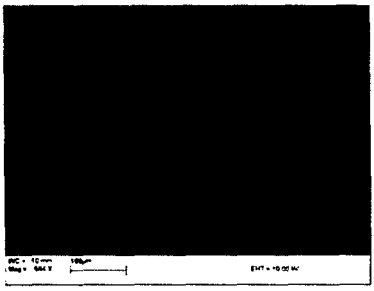
3.1 CA Membranes

In order to obtain continuous nanofibers, a minimum concentration of polymer solution is required. Below this, either droplets or combination of droplets and fibers will be observed. The reason for this is that under low concentration, the polymer chain entanglements might not be enough to make the jet stable to get the fibers [7]. Figure 2 shows the SEM images of different compositions of CA solutions. Only droplets of polymers were seen in the SEM image of the material with concentration of 7.5%. With the increasing concentration of the polymer solution, fibers were observed under the concentration of 10.0%, though droplets still existed. Above the concentration of 11.0%, three dimensional non-woven fibers were observed. The average diameters of the fibers obtained from electrospinning polymer solution increases with increasing concentration of the solution. However, when the concentration reaches 20.0%, the solution becomes too viscous to be electrospun. The diameters of fibers obtained varied from 100 nm to 1.2 μm while the average diameter was approximately 500 nm.

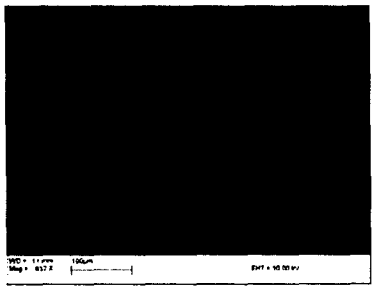
7.5%



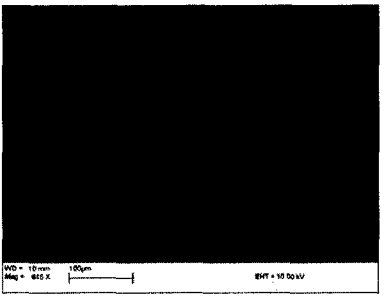
10.0%



11.0%



12.5%



15.0% (used for cell culture)

Fig.2. SEM images of different compositions of nanofibers.

3.2 Endothelial cell growth on membranes

Three materials were tested for their effect on endothelial cell viability: CA alone, CA+0.25%CT, CA+0.5%CT. Loose endothelial cells that could be easily washed off the matrix were tested with trypan blue, where dead cells appear blue and live cells appear clear. For all materials combined, 50-70% of the loosely attached cells were viable. CA alone (63±5%) was not different from 0.25% CT (65±4%), however, increasing CT concentration to 0.5% decreased viability by day 5 (58±3%*). Firmly adherent endothelial cells were tested using calcein/ethidium, where live cells appear green (due to calcein uptake and ethidium exclusion) and dead cells appear red (due to ethidium binding to nucleic acids). For all materials combined, total viability was ~90%. CT had no effect on the number of firmly attached *viable* cells. For each material, endothelial cells appeared to initially attach to single fibers and spread across fibers (Figure 3, left) and later form tubular-like structures (right).

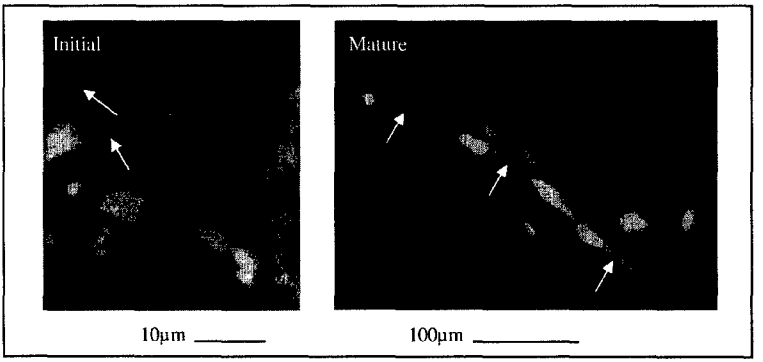


Fig.3. Human umbilical vein endothelial cells growing on CA+CT membranes. Green color indicates live cells; absence of red nuclei indicates no dead cells. Note endothelial cells growing along nanofibers (e.g., white arrow). [LEFT (40x): Blue is a false color due to phase contrast microscopy, optimized to view nanofibers. RIGHT (20x): fluorescence only]

Apparent porosity of the membranes was determined by the pressure required to push a known volume of fluid through a double thickness of membrane. The hydraulic conductivity was 1.8 x 10^{-1} cm^2 s g^{-1} for CA membranes and significantly less porous for CA+0.25%CT, 3.2 x 10^{-2} cm^2 s g^{-1} (5.6-fold difference). The higher concentration of CT (0.5%) was not different from 0.25%CT (3.0 x 10^{-2} cm^2 s g^{-1}).

4. Conclusion

This study shows that by means of electrospinning the CA solution, three-dimensional non-woven nanofibers membranes can serve as the scaffolds for vascular endothelial cell growth. The structure of the membranes that were produced mimic the topography and porosity of natural extracellular matrix. The presence of CT significantly decreases apparent porosity, perhaps by strengthening the material. CT had no effect on the viability of cells that were tightly adhered to the membrane. Over time, cells appear to form tubular-like structures similar in appearance to vascular capillaries.

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