Swelling, tensile testing, and SAOS-2 cell proliferation on nano-fibrous chitosan membranes.

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Statement of Purpose: Biodegradable scaffolds with nano-architecture may provide advantages over traditional guided tissue regeneration (GTR) membranes typically made of expanded polytetrafluoroethylene. Degradable materials do not require removal, but degradable collagen membranes are non-ideal and may degrade too quickly. Nano-structured materials are thought to more closely resemble the native extracellular matrix (ECM), and can convey nano scale forces to attached cells. Chitosan is a biodegradable biopolymer which is osteoconductive and has been previously electrospun into nano-fibrous membranes [1]. However, further characterization of these membranes is needed to link material properties and processing to biological responses. In this study, SAOS-2 osteoblastic cells were seeded on chitosan nanofibrous membranes and their proliferation was measured over 7 days. In addition, the swelling characteristics and mechanical properties of the membranes were measured.

Methods: The electrospinning procedure was adapted from Sangsanoh [2]. Briefly, 70 % deacetylated chitosan nanofibrous membranes were constructed using 6 wt% chitosan solutions in 70:30 v/v trifluoroacetic acid/methylene chloride solvent solution. After gently mixing for 24 hours, the solution was electrospun using a blunt 19G metal needle and a flowrate of 1 µL/min. The needle was connected to the positive electrode of the power source, while the target was connected to the ground. The voltage was set to 22.5 kV. The distance between the tip and the target was 16 cm. The whole apparatus was inside of a ventilated box, which was placed inside a fume hood. Once deposited and removed from target, the fiber mat was put under vacuum for 24 hours, then soaked in saturated Na_2CO_3 for 3 hours, then rinsed with deionized water until neutral. After drying, scaffolds were cut to size and sterilized using ethylene oxide gas. Swelling (n=4) was performed by placing the membranes in phosphate buffered saline for 45 minutes. Tensile testing (n=4) of dry dog-bone specimens was carried out using an Instron™ model 4465 and an extension rate of 1mm/min. SAOS-2 human osteoblastic cells were seeded on nanofibrous membranes or chitosan films made from acetic acid or tissue culture plastic. Scaffolds (n=3) were seeded at $1x10^4$ cells per mg scaffold. Cells were grown in McCoy's 5a medium supplemented with 10% FBS, and 1% Penn/strep. Proliferation was measured at days 3, 5, and 7 using Cell Titre Glo™ luminescent cell viability assay. Nanofibers were imaged using scanning electron microscopy (SEM) before and after neutralization to examine fiber morphology and dimensions.

Results: Swelling of the nanofibrous membrane was 149.8 ± 11.7%. Ultimate tensile strength was 11.6 ± 3 MPa. SAOS-2 cell number increased over the 7 days of culture (Figure 2). Initial cell attachment to chitosan films was greater than on the nanofibers but the amount of proliferation was not significant from day 3 to day 7.

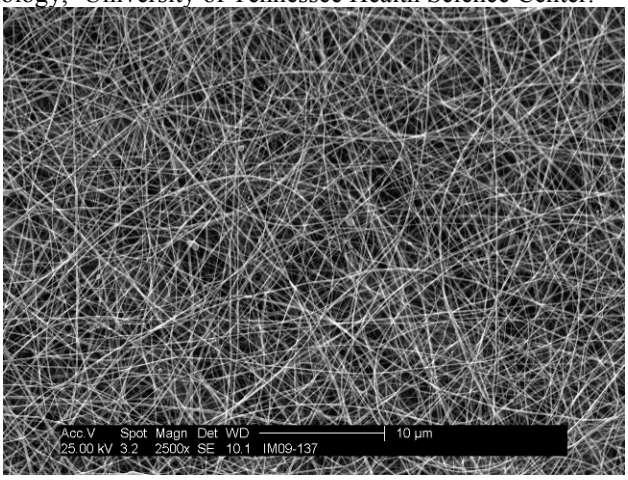

Figure 1 – SEM image at 2500X of nanofibers before neutralization.

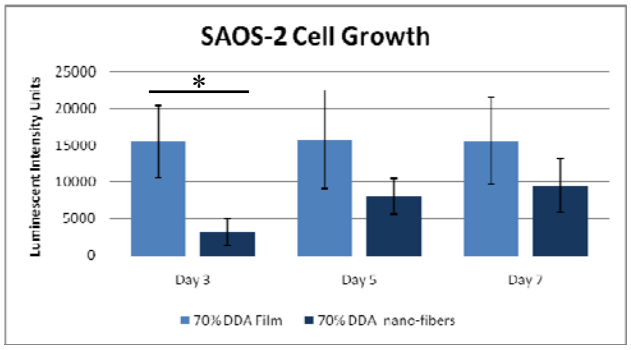

Figure 2 - Growth of SAOS-2 cells on nano-fibrous membranes versus chitosan films cast in acetic acid. More proliferation is seen on nano-fibrous membranes despite lower initial attachment (*$p<0.01$).

Discussion: Swelling of the nanofibrous mats in this study were about 50% higher than that reported by Sangsanoh. This may be due to the difference in DDA: 70 vs. 95%. Ultimate tensile stress of the neutralized dry membranes was higher than those reported for as-spun chitosan membranes, 11.6 versus 4.07 MPa [3]. This difference may be due to the neutralization of fibers causing fiber contraction and coalescence. Cell proliferation on nano fibrous membranes was better than that of chitosan films made from acetic acid, but a larger proportion of cells attached to the acetic acid films, causing a significant difference in groups only on day 3 ($p<0.01$). Future studies should include more detailed investigation of cell attachment and proliferation on nano-fibrous membranes as well as polymerase chain reaction studies to determine what genes, if any, are upregulated by the nanofibrous structure of the membrane.

Conclusion: This study shows that nano-fibrous chitosan membranes support bone cell proliferation and possess adequate mechanical strength for clinical applications.

References:

1) Ohkawa. *Biomacromolecules*. 7, 3291-3294.
2) Sangsanoh, P. *Biomacromolecules*. 7, 2710-2714.
3) Schiffman, JD. *Biomacromolecules*.8, 594-601.