

## Chemical modification of chitosan electrospun membranes for Guided Tissue Regeneration

Hengjie Su<sup>1</sup>, Daniel G. Abebe<sup>2</sup>, Kwei-Yu Liu<sup>2</sup>, Chaoxi Wu<sup>1</sup>, Gregory McGraw<sup>1</sup>, Tomoko Fujiwara<sup>2</sup>, Warren O. Haggard<sup>1</sup>, Joel D. Bumgardner<sup>1</sup>

<sup>1</sup>Biomedical Engineering Dept, <sup>2</sup>Chemistry Dept, The University of Memphis.

**Statement of Purpose:** Guided tissue regeneration (GTR) membranes are employed in dental/maxillofacial treatments combined with bone graft for regeneration of periodontal tissues lost to disease or injury. The membranes act as barriers to soft tissues and prevent their migration into the bone graft site. Chitosan membranes have shown promise in GTR applications due to their biocompatibility and degradability<sup>1</sup>. Electrospinning chitosan into nanofibrous membranes further adds biomimic extracellular matrix structure, and porosity to promote fluid/nutrient exchange between soft and hard tissue compartments while being cell occlusive. Using trifluoroacetic acid (TFA) we have electrospun chitosan membranes that exhibited in vitro and in vivo compatibility and degradability<sup>2</sup>. However, the nanofibrous structure is not optimal due to swelling of the fibers during removal of residual TFA solvent salts post spinning. Our goal was to evaluate a new process to eliminate TFA salts from the membrane without losing fibrous structure or compromising compatibility.

**Methods:** *Electrospinning:* Chitosan solution was prepared by dissolving 5.5%(w/v) Chitosan (71% DDA) in 70%(v/v) TFA and 30%(v/v) dichloromethane (DCM). Solution loaded 25kV voltage was electrospun with the pumping rate at 15 $\mu$ L/min. Membranes were collected by non-stick aluminum foil in the distance of 15 cm.

*Post-operation:* Electrospun membranes (n=4/treatment) were treated by 1] using saturated Na<sub>2</sub>CO<sub>3</sub> solution (control) or 2] our novel method based on using triethylamine (TEA) and tert-butyl dicarbonate (tbc). The TEA is used to extract the TFA salts while the tbc is used to protect the -OH groups on the chitosan to reduce fiber swelling and maintain nanofibrous structure.

*Characterization:* Morphology and diameter of fibers in membranes was examined by scanning electron microscope (SEM) and transmission electron microscope (TEM). Fourier transform infrared spectroscopy (FTIR) spectra used to evaluate the removal of the TFA salts and changes in chitosan chemistry pre- and post-treatments. SAOS-2 HTB-85<sup>TM</sup> osteoblast cells were seeded on both types of treated membranes (n=4/treatment group) and cell proliferation and viability evaluated over three days using the CellTite Glow<sup>®</sup> (Promega) and Live/Dead<sup>®</sup> stain (Molecular Probes) assays respectively.

**Results:** SEM and TEM examination showed that membranes treated by TEA/tbc exhibited more nano-scale fibrous structure than membranes treated by saturated Na<sub>2</sub>CO<sub>3</sub>. Fiber diameters of the TEA/tbc treated membranes ranged from 40nm to 130 nm (Fig.1) while fiber diameters were not able to be determined for the Na<sub>2</sub>CO<sub>3</sub> group. After immersion in PBS for 24 hours, membranes treated by TEA/tbc exhibited less than 30% swelling and retained their nanofibrous structure, compared with over 100% swelling of membranes treated by Na<sub>2</sub>CO<sub>3</sub>. FTIR spectra revealed that the three

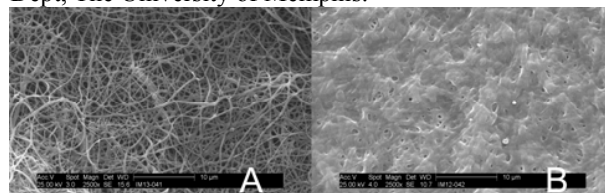


Figure 1. SEM (2500X) of membranes; A. treated by TEA/tbc; B. treated by saturated Na<sub>2</sub>CO<sub>3</sub> solution.

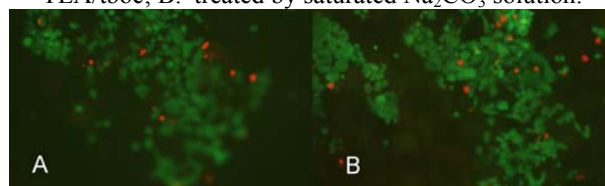


Figure 2. Observation from Live/Dead staining after cell culture for 3 days. Results were compared in between the membrane neutralized by A. TEA/tbc and by B. Na<sub>2</sub>CO<sub>3</sub>.

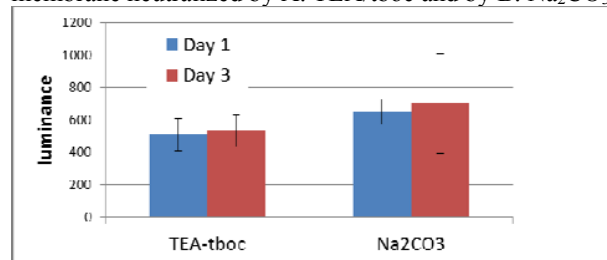


Fig 3. SAOS-2 growth on TEA/tbc and Na<sub>2</sub>CO<sub>3</sub> treated membranes.

transmittance peaks related to TFA salts at 720, 796 and 836 cm<sup>-1</sup> disappeared after the TEA/tbc treatment. Culture results revealed high viability of cells and similar cell proliferation on both types of treated membranes after three days cell culture (Fig.2 & 3).

**Discussion:** Chitosan membranes treated by TEA/tbc showed better nano-fiber morphology characteristics than membranes neutralized by saturated Na<sub>2</sub>CO<sub>3</sub> solution before and after being soaked in PBS. Retention of the nanofibrous structure for GTR applications may be of benefit for enabling nutrient exchange between soft gingival tissue and bone compartments and for mimicking the natural nanofibrillar components of the extracellular matrix during regeneration. The result of cell viability and proliferation showed that membranes treated by TEA/tbc had no toxicity to SAOS-2 cells. In future studies, suture pull out strength of these two kinds of membranes will be compared to identify the clinical handle ability. In vivo study will also be performed to further evaluate biocompatibility and function as a GTR membrane.

### References:

1. Norowski P, *et al.* J Tissue Eng Regen Med. 2012
2. Xu C, *et al.* J Biomed Mater Res B Appl Biomater. 2012

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