Electrospun Chitosan Guided Bone Regeneration Membranes for Delivery of Simvastatin to Stimulate Osteogenesis

Vishnu Priya Murali, Fernanda D. Guerra PhD., Tomoko Fujiwara PhD., Joel D. Bumgardner PhD.
University of Memphis, Memphis, TN

Statement of Purpose: Guided bone regeneration (GBR) membranes are used to augment healing by covering and protecting bone grafted spaces during the bone regeneration process and preventing soft tissue migration into the site. Electrospun chitosan membranes have a nanofibrous structure that mimics the native extracellular matrix. This supports cell attachment and growth and provides increased surface area for drug delivery. The porous structure of the membranes aid in the communication between osseous and epithelial tissue compartments and nutrient exchange while remaining cell occlusive, thereby serving as a good option for GBR membrane applications[1]. Simvastatin (SMV) is an anti-cholesterol drug that has been recently reported to promote bone growth and healing after local delivery, by antagonizing TNF-α inhibition of BMP-2, inhibiting osteoclast activity and improving angiogenesis[2]. This study examines the release of SMV from chitosan membranes subjected to four different treatments over a 28-day period and evaluates their cytocompatibility using mouse stromal cells.

Methods: *Electrospinning*: A 5.5 w/v% chitosan (71% DDA, 311kDa, Primex) solution was made in a trifluoroacetic acid (TFA): dichloromethane (DCM) (70:30 v/v) mixture and left to dissolve. Once most of the chitosan dissolved, the solution was filtered and electrospun at 27kV with the polymer solution flowing at 0.1ml/min. The distance between the syringe and the collector plate was ~15cm.

Post-spinning treatment: After electrospinning, the membranes were treated using 1) Fatty acids (acetic anhydride (AA), butyric anhydride (BA) or hexanoic anhydride (HA)) or 2) Triethylamine (TEA)-tert butyl dicarbonate (tBOC). These treatments were done to stabilize the chitosan fibers which tend to swell almost immediately when exposed to aqueous environment.

Elution Study: Small discs of treated membranes, 1cm in diameter and either 0.2mm (thin) or 0.7mm (thick) thickness, were loaded with 500, 250, 100 or 50µg SMV. The membranes were soaked in 500µl phosphate buffer saline (PBS) and incubated at 37°C. The PBS was collected and replaced over a period of 28 days. The collected samples were analyzed using Reverse-Phase HPLC (ThermoScientific Ultimate 3000).

Cytocompatibility study: W20-17, mouse stromal cells, were seeded at $1*10^4$ cells/well in 24 well transwells and grown for 1, 2 and 3 days with HA treated membranes loaded with no SMV or 50µg SMV. After each time point, the cell viability was analyzed using CellTiter-Glo® luminescent assay, which measures the amount of ATP present.

Results: From the HPLC analysis, it was found that for all the loadings and treatments (500, 250, 100 & 50µg) all the membranes showed a slow and sustained release of the drug until day 28 (Figure 1). The amount of drug released was not significantly different between the thin and the thick membranes ($p< 0.001$). For most of the groups, the tBOC treated membranes released SMV at a greater rate and the HA-treated released at the lowest. The differences in the release rates is thought to be due to the different mechanisms by which the hydrolyzed SMV interacts with the fatty acid chains or the tBOC groups. After 28 days, only the 50µg loaded tBOC and AA treated membranes seemed to have released 100% of the loaded SMV (Figure 1). The 100µg-loaded membranes released 40-80% of the drug depending on the treatments, whereas, the 500 and 250µg loaded membranes seemed to retain more than 50% of the drug for all the treatments. The HA treated membranes were used for cytocompatibility testing, since they released the lowest amounts of drugs and thus are expected to provide a sustained drug release for a longer time *in vivo*. It was found that the membranes with and without SMV were not cytotoxic and supported cell proliferation over the study period of 3 days (Figure 2).

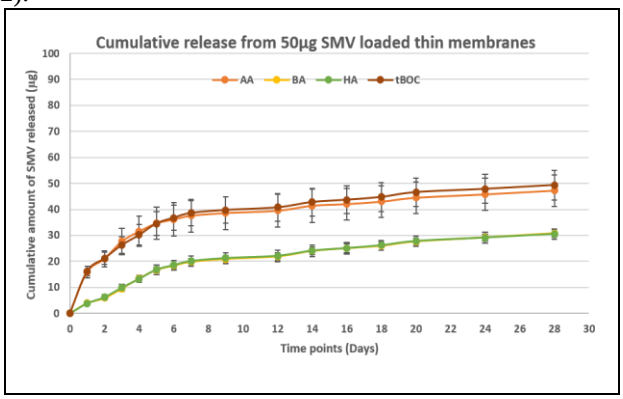

Figure 1: Representative cumulative release data of SMV from differently treated chitosan membranes

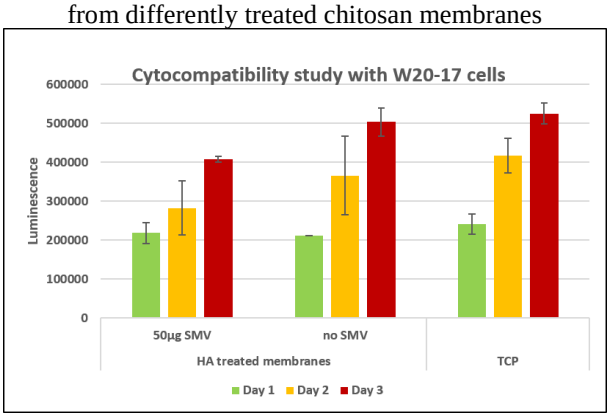

Figure 2: Cytocompatibility study with HA treated membranes using mouse stromal cells

Conclusions: The results indicate that SMV loaded electrospun chitosan membranes are cytocompatible and the type of post-spinning treatment and initial loading amounts can control the release pattern of the drug.

References: 1. Xu C, J. Biomed Mater Res B, 2012, 100B, 1435-1443

2. Ayukawa Y, Clin Oral Implants Res, 2004, 15.3, 346-350