Genipin-crosslinked nano-fibrous chitosan membranes: preliminary testing for use as GTR membrane.

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Statement of Purpose: Guided tissue regeneration (GTR) membranes are made of expanded poly-tetrafluoro-ethylene, are difficult to work with and require a painful removal surgery. Degradable materials do not require removal, but degradable collagen membranes have highly variant degradation kinetics and may degrade too quickly. Materials with nano-scale features are thought to more closely resemble the native extracellular matrix (ECM). Chitosan is a biodegradable biopolymer which is osteoconductive and has been previously electrospun into nano-fibrous membranes [1], however, improvements need to be made in the strength and handling characteristics for clinical applications. In this preliminary study, a natural crosslinking agent, genipin, is used to crosslink the membranes as they are electrospun. SAOS-2 osteoblastic cells were seeded on crosslinked and uncrosslinked chitosan membranes and their viability was measured over 5 days. In addition, the mechanical properties of the crosslinked and uncrosslinked membranes were measured in tensile tests.

Methods: The electrospinning procedure was adapted from Sangsanoh et al. [2]. Briefly, 70 % deacetylated chitosan was made into 5.5 wt% solutions in 70:30 v/v trifluoroacetic acid/methylene chloride solvent mixture. After gently mixing for 24 hours, the crosslinking agent, genipin, was added at a concentration of 5 or 10 mM. 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 a grounded rotating aluminum plate covered in non-stick aluminum foil. The voltage was set to 25.5 kV. The fiber orientation was random and the membrane was deposited with uniform thickness on the rotating target. The diameter of the fibers was previously shown to be 102 ± 34 nm. The fiber mat was put under vacuum overnight, then soaked in saturated Na_2CO_3 for 3 hours, then rinsed with water until neutral. After drying, membranes were sterilized using ethylene oxide gas. 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 uncrosslinked or genipin-crosslinked membranes or tissue culture plastic (TCP). Scaffolds (n=2) were seeded at $1x10^5$ cells per scaffold. Cells were grown in DMEM supplemented with 10% FBS, and 1% Penn/strep/Am-B. Cell number was measured at days 1, 3, and 5 using Cell Titre Glo™(Promega, WI, USA) luminescent cell viability assay. The viability and morphology of the cells was observed using Live-Dead stain (Invitrogen, OR, USA).

Results: SAOS-2 cell number increased on the chitosan membranes over the 5 days of culture (Figure 1). Ultimate tensile strength of the uncrosslinked membranes was 12.3 ± 4.9 MPa, the 5 mM crosslinked membranes was increased to 22.2 ± 6.7 MPa, and the 10 mM was increased to 32.2 ± 8.1 (p=0.012) (Figure 2).

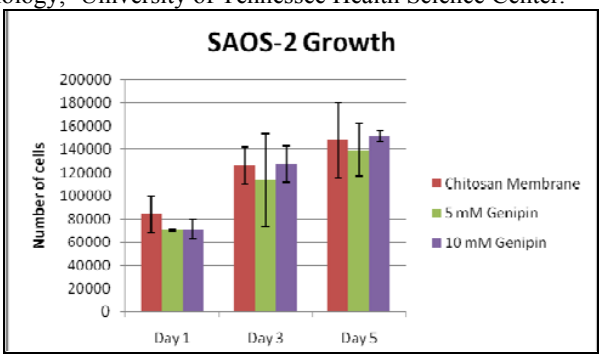

Figure 1 – SAOS cell number as determined by cell titre GLO (n=2, further measurements will increase n to 4)

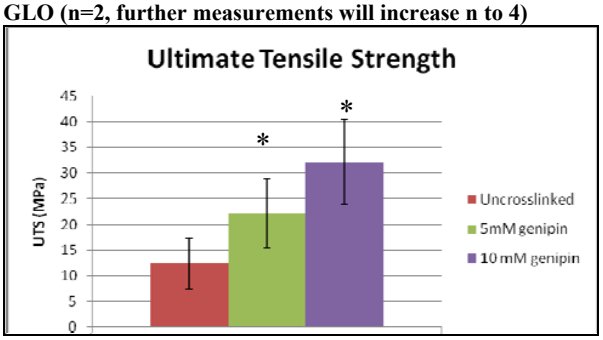

Figure 2 – Ultimate strength of membranes (n=4, n=3 for 10 mM, * indicates significance p<0.05)

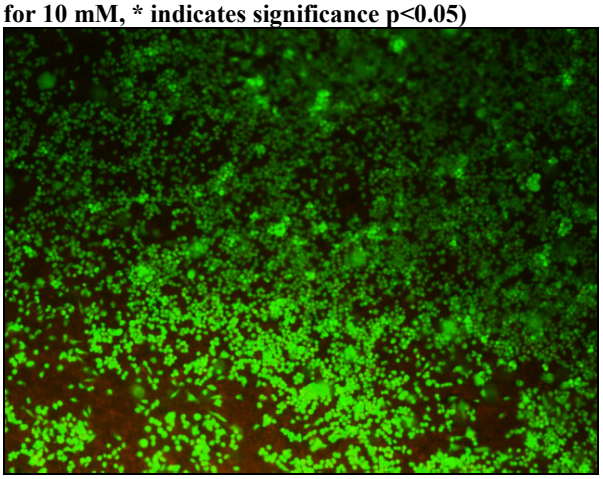

Figure 3 - Image of cells on 10 mM crosslinked membrane after 3 days of growth, note the large number of viable cells.

Discussion: The results show that genipin crosslinked membranes are not cytotoxic. SAOS cells proliferated on all three membranes and maintained a high level of viability over the 5 days (Figure 3). The tensile strength was also increased by crosslinking. Another advantage of crosslinking is that it might extend the biodegradation of the membrane to the clinician suggested 4-6 months.

Conclusion: Further studies will focus on the biodegradation kinetics of the membrane as well as drug/antibiotic-uptake and release.

References:

1) Ohkawa. *Biomacromolecules*. 7, 3291-3294.
2) Sangsanoh, P. *Biomacromolecules*. 7, 2710-2714.