Comparison of Electrospun and Lyophilized Gelatin + Chitin Whisker + Honey Membranes for Enhanced Periodontal Regeneration

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Statement of Purpose: Current biomaterials used as membrane barriers in extractions are often difficult to handle, degrade quickly, and offer no enhanced wound regeneration which is paramount for complete and timely closure of tissue over the bone graft. Therefore, our lab has developed two similar yet individually unique gelatin + chitin whiskers (CW) + Manuka honey (MH) compressed membranes which possess antibacterial and regenerative properties aimed to degrade within 6-12 weeks, allowing for retention of the graft while promoting a more rapid closure of the overlying tissue. One method utilizes electrospinning to create nanofibrous meshes while the other method involves fabrication of lyophilized sponges. Compressed membranes have increased handleability and are less porous. Less porous scaffolds are desired for this application to provide guided regeneration for tissue closure. Furthermore, it is documented that the addition of MH (antimicrobial by nature) can independently enhance the pro-regeneration response. CW, a novel nanofiller, have been shown to reinforce both synthetic and natural polymeric structures and exhibit biocompatibly and biodegradability. This abstract highlights the similarities and differences between the two different membranes and addresses the clinical advantages over current products.

Methods: All CW were prepared according to Dufresne's method with minor modification [1], and all solutions were incubated at 37°C overnight allowing gelatin and MH to completely dissolve. For electrospun meshes, CW (15 wt% of gelatin) were redispersed in 2,2,2-trifluoroethanol (TFE) by ultrasonication. Gelatin (Type B) at 140 mg/mL and MH (0-10 wt% of gelatin) were added to the solution and electrospun at optimal conditions. Meshes were crosslinked after compression using 40 mM 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide) (EDC) for 21 hours at room temperature. For sponges, CW (10 wt%) were redispersed in deionized water by ultrasonication. Gelatin at 30 mg/mL and MH (0-25 wt%) were added to the CW solution. 40 mM EDC was quickly mixed with the solution after incubation, then frozen and lyophilized. Both membrane types (4 layers for meshes and 4 mm thick slices for sponges) were compressed using a hydraulic press. All membranes were characterized for: degradation (BCA assay), mechanical properties (uniaxial and compression testing), and clinical formability (examined by oral surgeon). Membranes were cultured with human dermal fibroblasts (HDFs) for 14 days and cell adhesion (fluorescent staining) was examined.

Results: Scanning electron microscopy (SEM) was used to image compressed and non-compressed meshes and sponges (Figure 1). For electrospun meshes, hydrated membranes with 0% and 10% MH possessed the best clinical formability compared to CollaPlug ® (collagen membrane, does not hold shape). Formability can be tailored by increasing or decreasing the number of compressed layers. Compression of meshes retained fibrous architecture though some fiber welding was noticed post-compression, most likely dependent on the crystallization state of the MH. Cells were visible on the surface of all MH meshes. Degradation and mechanical testing are currently ongoing though preliminary work suggests that higher MH concentrations increase Young's modulus due to the presence of more crystalline MH. For sponges, hydrated membranes tore easily and handled similarly to clinical collagen membranes. However, dry membranes had greater handleability compared to controls. Methods to increase formability will be explored. Sponge compression resulted in a less porous membrane while providing a bioactive surface. Increased cell attachment was seen on day 1 MH membranes and KLS Martin ® (polylactic acid film) compared to 0% MH and Bio-Gide ® (collagen) membranes. Studies suggest degradation rate is tailorable based on varying MH concentrations. Compression testing showed that all sponges behave similarly and can retain mechanical integrity during testing (unlike Bio-Gide). Future studies will analyze cell proliferation, secreted regenerative markers, and extracellular matrix production.

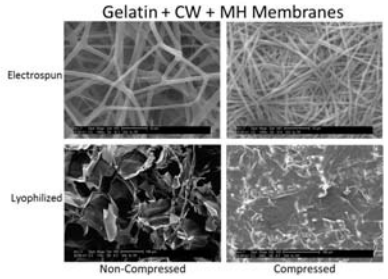

Figure 1. SEM images of the two different membranes.

Conclusions: Hydrated meshes and sponges have improved and similar formability compared to collagen clinical controls, respectively. Fabrication of membranes can be tailored to improve formability as needed. Both meshes and sponges exhibited improved cell adhesion in membranes with MH as compared to 0% MH and collagen clinical controls. Although KLS Martin also showed significant cell adhesion, films such as these are not desirable as degradation leads to an acidic microenvironment. Gelatin + CW + MH membranes are biocompatible and degrade without releasing harmful byproducts into the surrounding environment. Both meshes and sponges with the addition of MH are shown to enhance cell response, and therefore potential for guided tissue regeneration which is the ultimate goal of both membranes.

References: 1. Ji, Y-L, *et al. Carbohydrate Polymers*, 87, 2313-2319, 2012