Evaluation of glutaraldehyde crosslinked electrospun chitosan membranes modified with gelatin and elastin for skin wound healing

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Statement of Purpose: Electrospun, chitosan membranes (ESCM) have seen success for guided bone regeneration applications [1], [2]. Chitosan is a biodegradable, naturally occurring polysaccharide derived from crustacean exoskeleton that has many pro-healing properties applicable to other tissue. This biomaterial can also be mixed with other polymers, like gelatin and elastin, to improve mechanical properties and bioactivity, increasing its healing capabilities [3]. Specifically, the gelatin/elastin-polysaccharide nanofiber structure may serve as a template in skin tissue engineering applications. However, when untreated, the chitosan-elastin membrane's nanofiber structure is lost in aqueous environments leading to poor cytocompatibility due to a lack of extracellular matrix mimicking fibers. This has led researchers to develop post treatments to retain fibrous morphology including amine group neutralization, hydrophobic treatments, and crosslinking. Glutaraldehyde has been used to crosslink electrospun fibers successfully while retaining nanofiber morphology [4]. This study explores the usage of glutaraldehyde as a crosslinker to prevent the loss of fibrous morphology of chitosan-gelatin/elastin membranes in aqueous environments.

Methods: ESCM fabrication was performed through electrospinning of chitosan solutions. The groups of solution tested were chitosan (C), chitosan with 10w/w% elastin (C10E), chitosan with 40w/w% elastin (C40E), and chitosan with 45w/w% gelatin (gel-C). All membranes were spun using a trifluoroacetic acid (TFA) and dichloromethane (DCM) solution at a ratio of 7:3 TFA:DCM respectively. After fabrication of electrospun membranes, samples underwent a triethylamine wash to remove residual TFA salts from the spinning process. From this point, all sample groups were treated utilizing glutaraldehyde vapor deposition for 24 hours to crosslink the membranes. Following the crosslinking, samples were washed in sodium bisulfite for 24 hours to neutralize any residual glutaraldehyde vapor. Membrane characterization was performed across all sample groups, including scanning electron microscopy for fiber diameter and morphology, and swelling ratio. Cytocompatibility was evaluated by seeding membranes with adipocyte-derived stem cells (ASC), immortalized human keratinocytes (HaCaT), and normal human dermal fibroblast (NHDF) cells for 72 hours. Quantification was established using a resazurin fluorescence assay and all results normalized TCPS control for each cell type.

Results:

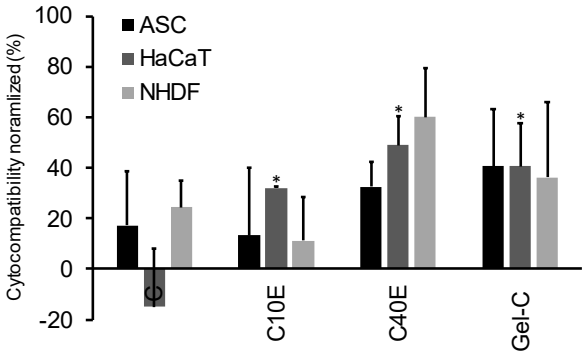

Figure 1. Cytocompatibility of glutaraldehyde-crosslinked membranes with ASC, HaCaT, and NHDF cells after 72-hour culture.

Fiber characterizations showed all membrane groups were able to retain nanofiber morphology after time in aqueous environment indicating successful crosslinking (data not shown). A two factor ANOVA was conducted on cytocompatibility results and found a significant interaction between membrane types ($p = 0.0002$) and no interaction between cell types ($p = 0.5$). Further one factor ANOVA testing for each cell type found differences in HaCaT results ($p = 0.0019$), but not ASC ($p = 0.1$) or NHDF ($p = 0.12$). Further analysis on HaCaT results found significant differences in C10E ($p = 0.015$), C40E ($p = 0.0017$), and gel-C ($p = 0.004$) when compared to their unmodified C counterpart.

Future work: Future characterization includes crosslinking degree, degradation rates, and mechanical properties. Also, more studies to optimize fabrication, treatment, and processing of material will be studied. Finally, angiogenic potential for wound healing will be assessed *in vitro*.

References:

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