Cellular response of kidney cells on chitosan based scaffolds

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Statement of Purpose: Diabetes is a rapidly growing health problem throughout the world that results in diabetic nephropathy and end stage renal disease. It affects blood glucose concentration by means of insulin resistance. It is believed that prolonged levels of hypo- and hyperglycemia impact the deposition of ECM proteins laminin and fibronectin via the protein kinase A catalytic pathway. Though cells have been cultured in 2D dishes since its inception, 3D cell culturing provides more faithful replication of *in vivo* data, better transport phenomena for growth factors, and restoring physiological cell to extracellular matrix (ECM) interactions. Chitosan is a natural polysaccharide derived from chitin that when cross-linked with its derivative, carboxymethyl chitosan (CMC), they form an ionic bond that aids in scaffold stabilization. The objectives of this study were to create three-dimensional chitosan-based scaffolds that were suitable for cell culture and to evaluate cell proliferation and protein expression of fibronectin in glucose-treated mesangial cells, cells around blood vessels in the kidneys.

Methods: Acetic acid, chitosan, ethanol, phosphate buffered saline, and sodium hydroxide were purchased from Fisher Scientific (Waltham, MA). CMC was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Dulbecco's Modified Eagle Media was purchased from ATCC (Manassas, VA). Rabbit IgG was purchased from Bio-Rad. Antibodies were purchased from ABCAM (Cambridge, UK). Glucose and radioimmunoprecipitation assay (RIPA) buffer were purchased from Sigma Aldrich (St. Louis, MO). Chitosan and CMC were prepared in 4, 5 and 6 % solutions. They were centrifuged in 1:1, 3:2 and 3:1 ratios, frozen and lyophilized in order to create porous scaffolds. These scaffolds were stabilized using sodium hydroxide and sterilized using sequential ethanol washes. Renal mesangial cells were seeded using Dulbecco's Modified Eagle Media and treated with glucose in order to simulate hypo- and hyperglycemia. RIPA buffer was used to extract the cells from the scaffolds followed by Western blot analysis.

Results: The 5% 1:1 chitosan : CMC scaffolds showed the best cross-sectional porosity when compared to its 3:2 and 3:1 counterparts. It displayed micro-pores throughout the entire scaffold, promoting cell motility, adhesion and proliferation. Glucose-treated cells seeded in the scaffolds adhered and proliferated to the surface. Extracted proteins exhibit similar characteristics to those shown *in vivo*.

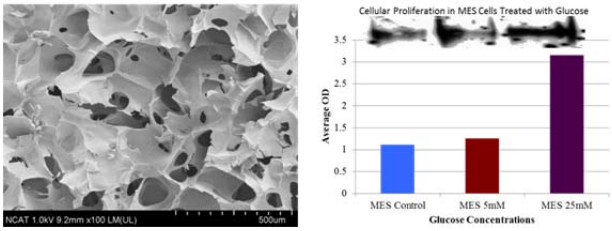

Figure 1 (A-B). (A) denotes a SEM image of a lyophilized 5% 1:1 chitosan: CMC scaffold. (B) shows fibronectin protein expression expressed as the average optic density in 48 hours by mesangial cells as a function of control, low and high glucose treatment concentrations. ECM fibronectin increases over a 48 hour period in high glucose conditions, meaning prolonged hyperglycemia increases the amount of protein over time

Conclusion: Chitosan-based scaffolds exhibit the biocompatible characteristics required for successful cell adhesion and proliferation. The 5% 1:1 chitosan : CMC ratio demonstrated the best porosity and motility capabilities. The fibronectin probed showed *in vivo* characteristics of hypo- and hyperglycemic individuals.

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