Improved Mesenchymal Stem Cell Proliferation
on Glycosaminoglycan Immobilized Chitosan - Effects of Membrane Thickness

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Statement of Purpose: Glycosaminoglycans (GAGs) have roles in cell signaling, migration and differentiation either by direct-receptor interactions or by modulating growth factor trafficking and matrix organization. Chitosan is a natural polysaccharide that is biodegradable and biocompatible. In previous work, chitosan was used as a substrate for covalent immobilization of GAGs, and studies were performed to investigate the effects of GAG immobilization on the proliferation and differentiation characteristics of mesenchymal stem cells (MSCs). During those cultures it was observed that MSCs preferentially attached to the thicker parts of the membranes. In this study, we have investigated MSC proliferation as a function of the membrane thickness and characterized chitosan films in terms of protein binding and membrane microstructure.

Methods: Chitosan membranes were cast into 24-well culture plates by air-drying 1.5 wt% chitosan solutions in acetic acid to deposit 0.375, 1.875 or 3.75 mg/cm^2. Cast membranes were neutralized and GAGs were immobilized on the membranes using carbodiimide (EDC) chemistry at a GAG to chitosan ratio of 1 mg/mg. GAGs studied included heparin (HEP), heparan sulfate (HS), chondroitin 6-sulfate (C6S), chondroitin 4-sulfate (C4S), dermatan sulfate (DS) and hyaluronic acid (HA). Adult rat bone marrow MSCs at passage number 3 were seeded onto GAG-chitosan membranes at a density of 5,000 cells/cm^2 in DMEM with 10% FBS. Cell proliferation was assessed by the measurement of the MTT reducing activity of the cells on days 2 and 4. Adsorption of serum fibronectin and vitronectin, on GAG-immobilized membranes was also evaluated by an ELISA-based method. Wide-angle X-ray scattering (WAXS) spectra of chitosan membranes was used to characterize microstructure.

Results / Discussion: It was found that in the absence of the cells chitosan membranes produced a reduction of MTT in proportion to the amount of chitosan present, and the effect was independent of the MTT solvent. Although analysis of this observation is incomplete, the results suggest that chitosan films contain an enzyme-like activity capable of reducing MTT. This phenomenon is currently being studied further. MSCs were cultured on GAG-immobilized chitosan membranes of different thicknesses. Images from C6S-immobilized surfaces (Figure 1) show that the extent of cell spreading increased considerably with membrane thickness. It was also found that the MSC growth rate increased with membrane thickness for all the GAGs studied (Figure 2). However on heparin, the increase exhibited saturation beyond 1.875 mg/cm^2. Examination of the relative binding levels of serum fibronectin and vitronectin on GAG-immobilized chitosan membranes showed that vitronectin adsorption on the surfaces increased with chitosan membrane thickness while fibronectin binding level on exhibited no clear trend.

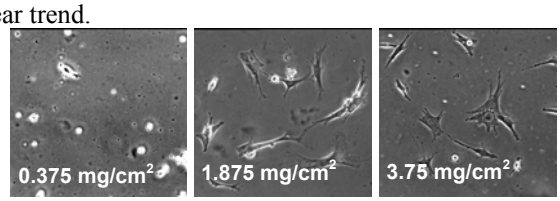

Figure 1 - MSCs on C6S-immobilized chitosan spread more on thicker membranes.

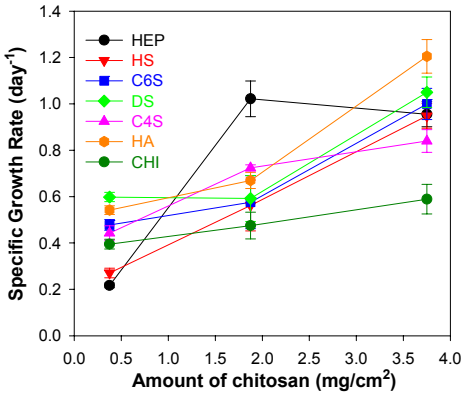

Figure 2 - Effect of membrane thickness on the MSC proliferation rates

WAXS (Figure 3) spectra showed the appearance and subsequent broadening of a peak at 2θ = 26.34° as the membrane thickness was increased from 1.36 to 5.45 mg/cm^2. This suggests an increase in the amorphous fraction with increasing thickness. The less crystalline structure of the membranes at higher thicknesses may explain the increased level of cell spreading and growth rates observed in these membranes.

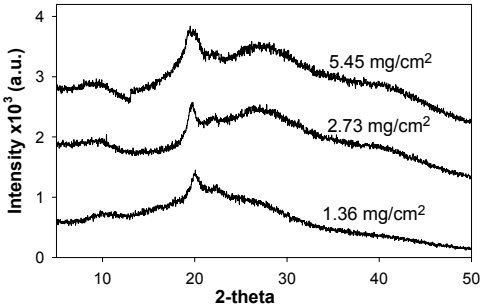

Figure 3 - WAXS patterns of chitosan films

Conclusions: Chitosan was found to have MTT reduction activity in the absence of cells. The MSC proliferation appeared to correlate with the level of vitronectin bound on the membranes. Improved cell interactions with the surface on thicker membranes may also be explained by the changes towards a less crystalline membrane microstructure as membrane thickness increased.