Immobilized Glycosaminoglycans Modulate Proliferation and Organization of Cultured Cholangiocytes

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Statement of Purpose: Cholangiocytes (bile duct epithelial cells) are an important functional component of the liver. They perform major biological functions in formation and transport of bile, as well as producing cytokines and chemokines. Current liver tissue engineering efforts focus on factors that regulate primary hepatocyte function. Less attention has been paid to the cholangiocyte component, and the incorporation of biliary excretion into tissue engineered liver designs. Success in engineering a functional bile drainage system would represent substantial progress towards a fully functional artificial liver. However, information on interactions of cholangiocytes and biomaterials is very limited. We propose to develop a liver scaffold based on chitosan and glycosaminoglycans (GAGs) in which chitosan will act as the main structural component. GAGs are a family of complex polysaccharide components of the extracellular matrix (ECM) that interact with a variety of ECM proteins, growth factors, and cell receptors. As a result they are capable of modulating diverse cellular functions including enzyme activity, growth factor signaling, angiogenesis, and differentiation. In this study we investigated cholangiocyte behavior, in response to culture on GAG-crosslinked chitosan materials. The work aimed towards identifying material formulations capable of modulating cholangiocyte organization and growth with the long term goal of engineering liver tissue with functional bile ducts.

Methods: Chitosan membranes were prepared by casting and air drying chitosan solutions (1.5 wt% in 0.2 M acetic acid) in 24-well plates and neutralized with 0.2 M NaOH. GAGs were then immobilized on the chitosan membranes by covalently linking the GAG carboxyl groups to the free amine groups on chitosan using carbodiimide chemistry. The GAGs studied were: heparin (Hep), hyaluronic acid (HA), dermatan sulfate (DS) and chondroitin-4-sulfate (C4S), chondroitin-6-sulfate (C6S) and carboxymethyl dextran sulfate (DXS). A GAG to chitosan mass ratio of 0.33 was used in all cases. Toluidine blue staining was employed to verify the stability of the immobilized GAGs surfaces. Normal rat cholangiocytes (a gift from Dr. Nicholas F. LaRusso at the Mayo Clinic, Rochester, MN) were seeded onto surfaces at a density of 4000 cells/cm^2. Cholangiocyte growth medium (Vroman B and LaRusso NF. Lab Invest. 1996; 74 (1): 303-313.) was changed 24 h post-seeding and every 36 h thereafter. Cell morphologies were monitored using phase contrast microscopy. Morphometric analyses were conducted using image analysis software (Sigma Scan Pro, Systat Software Inc., CA). Cell growth rates were determined by the XTT-formazan conversion assay.

Results/Discussion: Cholangiocytes attached to all test surfaces. However, large differences in cell morphology were observed on various GAG-chitosan surfaces. Cholangiocyte cells appeared to be extending several narrow pseudopodal structures from a central cell body at the edge of cell aggregates on all GAG-chitosan surfaces. Cells exhibiting a cuboidal morphology coexisted with those exhibiting a stellate morphology on the same surface. Tubule-like structures were formed on several GAG surfaces (Figure 1). On DS, C6S and DXS, cells spread enough to establish a cellular network. In contrast, on HA, cells formed three-dimensional cyst-like structures. The specific growth rates appeared to correlate with the cell spreading results. Specifically, the highest growth rates were observed on those surfaces which also produced the highest cell spreading.

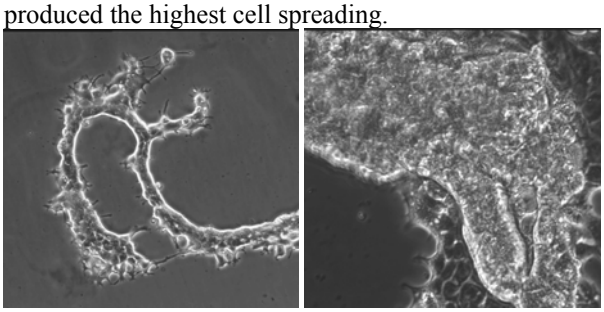

Figure 1: Cholangiocytes on polysaccharide surfaces.

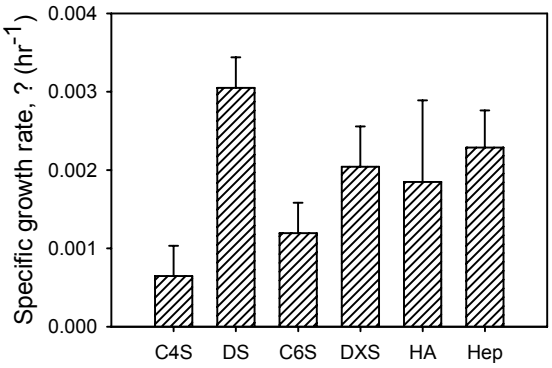

Figure 2: Specific growth rates of cholangiocytes on immobilized GAGs.

Conclusions: These results indicate that immobilized GAG-chitosan materials can be used to modulate the growth and three-dimensional organization of cholangiocytes in vitro. The fact that various GAG-chitosan materials support different rates of proliferation and organization suggests that they are useful tools in the design of scaffolding materials for engineering liver tissue with functional bile ducts.