495d Morphological and Functional Responses of Hepatocytes Cultured on Glycosaminoglycan-Chitosan Membranes

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Successful engineering of liver tissue requires scaffold systems that facilitate hepatocyte organization and function. In this study, the effects of immobilized glycosaminoglycans (GAGs) on the morphology, dynamic organization and metabolic function of primary hepatocytes were evaluated. Chitosan membranes were cast into culture dishes and modified by covalent linkage of various GAGs via carbodiimide chemistry. Binding of fibronectin and vitronectin onto these surfaces from serum-supplemented medium was quantified via ELISA. GAG-chitosan membranes were seeded with rat hepatocytes, and cultured for 7 days. Hepatocytes cultured in the collagen-sandwiched monolayer configuration served as controls. Cell morphology and the dynamics of cellular assembly on the GAG-chitosan membranes were assessed by time-lapse video microscopy. In addition, liver-specific function was assessed by measuring rates of urea and albumin synthesis. On immobilized GAG surfaces, hepatocytes rapidly formed attached spheroids 119 to 168 microns in diameter. Hepatocyte functions were maintained over the culture period with heparin surfaces exhibiting functional levels comparable to the control collagen sandwich cultures. Overall, the results indicated that GAG-chitosan matrices are a suitable substrate for hepatocyte scaffold culture and promote rapid formation of spheroids that maintain extended in vitro function. While hepatocyte functions varied with GAG type, the results did not correlate directly with levels of bound serum proteins, thus suggesting a role for other GAG-influenced signaling pathways. A discussion of possible mechanisms for these effects and their significance for the engineering of functional liver tissue in vivo will be presented.