Morphological and Growth Responses of Vascular Smooth Muscle Cells Cultured on Immobilized Heparin and Dextran Sulfate Surfaces

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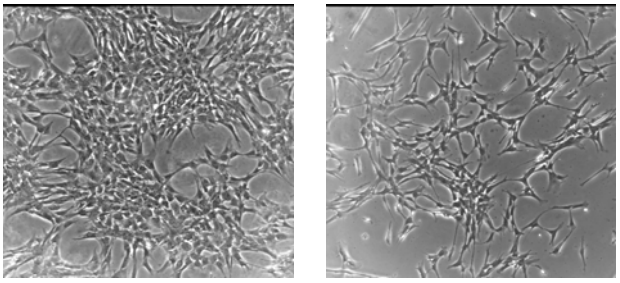
Purpose: Congenital diseases of the heart valves and large arteries (e.g. the aorta) are significant causes of morbidity and mortality among children. Because immunological concerns prevent replacement of the affected structures with donated living tissue, most of these problems require surgery to implant artificial structures or non-living tissue. However with current standard practices, multiple open-heart surgeries are required prior to adulthood to allow installation of larger conduits. The use of living vessel and valve tissue that can grow, remodel and adapt as the child grows would eliminate most of the need for repeated surgeries. The tissue engineering approach to organ repair and regeneration offers the possibility of synthesizing such tissue structures using cells derived from the patient. Thus the long-term goal of this work is the development of methods for generating transplantable vascular tissue (vessels and valves) for pediatric applications.

A successful tissue engineered vascular graft requires a material that will support the proliferation and function of vascular smooth muscle cells (SMC), since these cells comprise the largest percentage of cells in vascular tissue. Glycosaminoglycans (GAGs) influence cell growth, act as tissue organizers, and modulate activities of many growth factors including those involved in the formation and growth of blood vessels. Chitosan is a biodegradable and biocompatible polysaccharide that is being studied as a structural scaffolding material for tissue engineering. In some applications it is blended with collagen to enhance its cell adhesion properties and degradation kinetics. In this work, we compared the effects of a GAG and a GAG-like polyanion as modifiers of SMC activity. Specifically we evaluated the effects of heparin and dextran sulfate immobilized on chitosan films, on the morphology and proliferation of SMC.

Methods: Membranes of chitosan and chitosan-collagen (90/10 mass ratio) were cast from solution and modified by covalently attaching various amounts of heparin or dextran sulfate using carbodiimide crosslinking. The GAG to chitosan mass ratios employed were 0.08, 0.2 and 1. In order to explore the role of serum adhesion proteins in modulating the GAG-SMC interaction, binding of fibronectin and vitronectin to the GAG-modified membranes was quantified with an immunoassay. The GAG-modified membranes were also seeded with porcine smooth muscle cells at a density of 6000 cells/cm^2 and cultured for 4 days. Cell morphology was assessed by phase contrast microscopy and quantitative image analysis, and cell proliferation rate was measured by the XTT-formazan assay.

Results and Discussions: Immobilized heparin membranes showed the highest fibronectin absorption at the intermediate GAG-chitosan ratio of 0.2. For dextran sulfate, the amount of fibronectin adsorbed on the membranes decreased with increasing surface density of immobilized GAG. In contrast, vitronectin showed a higher absorbance on GAG-chitosan-collagen membranes. The phase contrast microscopy and XTT results confirmed that SMC grew fastest on the highest immobilized GAG levels. Comparing the polyanions, cells proliferation rates were substantially higher on dextran sulfate as opposed to heparin.



(a) **(b)**

Figure: Porcine aortic smooth muscle cells after 4 days of culture on covalently immobilized (a) Dextran sulfate-chitosan membrane; and (b) Heparin-chitosan membrane. Both polyanions were immobilized at GAG/chitosan ratios of 1 mg/mg.

Previous results indicated that ionically immobilized heparin inhibited SMC proliferation while proliferation was promoted by ionically immobilized dextran sulfate (Chupa, J.M. et al. Vascular Cell Responses to Polysaccharide Materials, Biomaterials 2000, 21: 2315-2322). This study suggests that covalent immobilization reverses the heparin inhibition of SMC proliferation, but immobilized dextran sulfate species still retain proliferative superiority. Proliferation results did not directly correlate with relative levels of fibronectin or vitronectin binding, suggesting that receptor or growth factor interactions of the immobilized species may be key. Evaluation of SMC responses to other covalently immobilized GAGs is underway and results will be reported.

Conclusions: Our results indicate that covalently immobilized heparin and dextran sulfate are suitable culture substrates for vascular SMC and thus may be useful components of polysaccharide-based, tissue engineered cardiovascular structures.