SMALL DIAMETER VASCULAR GRAFT TISSUE ENGINEERING AND BIOREACTOR TECHNOLOGY

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INTRODUCTION

Advancements in bioreactor technology have only recently been acknowledged as an integral part of tissue-engineered product development [1-4]. Bioreactors should provide important biochemical and mechanical stimuli for directed *in vitro* tissue growth to meet patient demand. Key bioreactor design objectives include reactor scalability, product reproducibility, and processing flexibility to grow tissues with characteristics appropriate for human implantation in a cost- and time-efficient manner.

In vascular tissue engineering, the goal is to provide a functional living artery composed of cells and biologic matrix as an off-the-shelf product for replacement of diseased small-caliber arteries. This project aims to develop a scaleable perfusion bioreactor system for the generation of small diameter tissue-engineered vascular grafts. Specifically, we have focused on seeding biodegradable polymeric scaffolds with smooth muscle and endothelial cells under dynamic conditions and providing physiologically relevant mechanical and biochemical stimuli that increase matrix synthesis and mechanical strength. The bioreactor is composed of compact, modular units with independent lumenal and external flows that can be assembled in series and used with various cell-scaffold combinations (Figure 1). Multiple constructs can be seeded and grown simultaneously in a well-regulated environment that promotes arterial construct maturation and differentiation. A wide range of flow rates and pressure waveforms can be used to mechanically precondition the constructs in the bioreactor's homogeneous biochemical environment that supports long term aseptic growth.

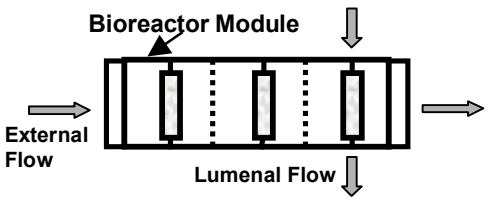

Figure 1. Bioreactor schematic

MATERIALS AND METHODS

Bovine aortic smooth muscle cells (SMC) were isolated from newborn calves and used at low passage to seed polyglycolic acid (PGA) non-woven felts. The tubular scaffolds were prewetted in supplemented culture medium overnight, mounted in the bioreactor, and seeded with SMC both through the lumen and on the external surface by a dual syringe pump under dynamic conditions. A peristaltic pump (operating at 1.5 Hz) was then used for mechanical stimulation (lumenal flow) and nutrient perfusion (external flow). After the SMC layer was cultured for at least 2 weeks, the lumen of the constructs was dynamically seeded with bovine aortic endothelial cells by a dual syringe pump. Arterial constructs were grown for up to 35 days and analyzed for DNA, collagen, and elastin synthesis by biochemical assays, immunohistochemistry and histology. Cell distribution and polymer degradation were visualized by scanning electron microscopy and media samples were taken throughout the experiments to quantify cellular metabolic activity by measuring pH, and glucose/lactate. Cell differentiation was evaluated by immunohistochemical analysis using appropriate phenotypic markers.

RESULTS AND DISCUSSION

Cell proliferation data from one-module experiments showed an over 3-fold increase in cell number in 4-, 9-, and 16-day experiments. Smooth muscle cells were uniformly distributed across the thickness of the constructs and collagen deposition was evident within 4 days. Longer-term experiments of up to 35 days resulted in an over 3-fold increase in cell number and enhanced matrix deposition. Collagen synthesis was increased compared to short-term experiments and amorphous elastin deposition was observed within 5 weeks of bioreactor culture. The independent lumenal and external flow loops of the bioreactor allowed the comparison of two different cell-seeding protocols. When cells were seeded only through the construct lumen, they populated the lumenal and external regions of the wall and led to the creation of a cell-depleted region in the middle (Figure 2A). However, when the smooth muscle cell suspension was seeded both on

the lumenal and on the external surface of the constructs, cells were uniformly distributed across the wall (Figure 2B).

Of further note, smooth muscle cells were in a contractile phenotype and expressed smooth muscle α-actin, mostly in the lumenal and external surface regions. The smooth muscle α-actin expression was upregulated compared to SMC monolayer cultures indicating that the mechanical and biochemical stimuli can shift the smooth muscle cells towards a more contractile phenotype. Results of lactate and glucose assays and pH measurements demonstrated that smooth muscle cell growth in the polymer scaffold was not metabolically compromised during the experiments. In addition, the polyglycolic acid fiber diameter decreased by 38% within 35 days of culture, and fibers were degraded into small fragments. Cells were attached and spread on the fibers and formed an extensive extracellular matrix network of collagen and elastin fibrils. This result indicates that the polymer scaffold does not contribute to the construct's mechanical strength to a large extent after 35 days in the dynamic environment of the bioreactor.

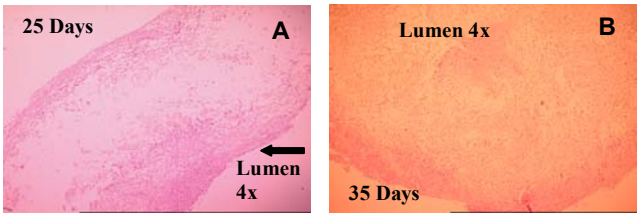

Figure 2. Smooth muscle cell distribution across construct wall after 25 (A) and 35 (B) days in bioreactor culture

Monitoring of the time-averaged flow rates for both the lumenal and the exterior inlet and outlet showed large variations the first days of culture, which were subsequently reduced significantly (Figure 3). This outcome indicates cross-flow through the scaffold wall during the first two weeks in culture, which is then reduced as the scaffold is populated with smooth muscle cells and their matrix, and permeability is decreased.

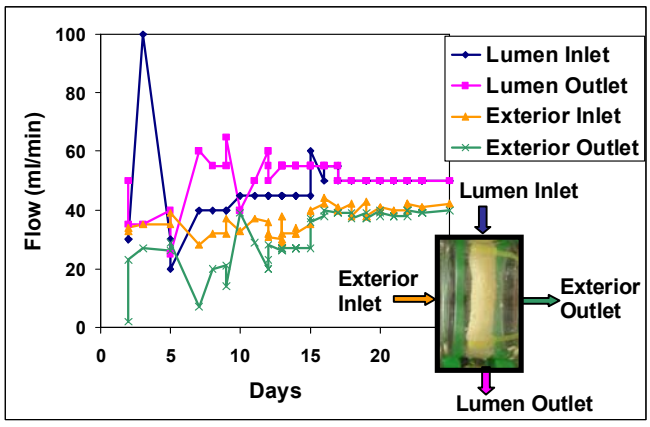

Figure 3. Flow rate monitoring in the bioreactor (flows are referenced with respect to the construct shown in the Figure)

A three-module bioreactor experiment was designed to assess seeding uniformity and reproducibility during multiple construct growth. Cells proliferated with similar rates among all constructs, were uniformly distributed, and aligned in the flow direction, showing that this bioreactor system can be used to generate several arterial constructs reproducibly.

CONCLUSIONS

The perfusion bioreactor system discussed in this work promotes rapid cell growth and extracellular matrix deposition under pulsatile flow conditions. It can be used to dynamically seed cells on tubular scaffolds both through the lumen and on the external surface to achieve uniform cell distribution, which results in uniform matrix deposition and comparable mechanical properties across the construct wall. In addition, its independent lumenal and external flow loops can be used for the mechanical stimulation of the constructs with pulsatile shear stress through the lumen while the external flow provides nutrients. The controllable environment of the bioreactor promotes smooth muscle differentiation and the creation of a smooth, confluent endothelium. Finally, monitoring of cross-flow through the construct wall is indicative of wall permeability and can be used as a maturation marker for the growing vascular tissue.

The bioreactor can accommodate the seeding and growth of several constructs in the same unit under sterile conditions and can be used to investigate the properties of different cells and scaffold materials. Therefore, this bioreactor is a useful tool towards the development of precise quantitative relationships between construct environment and artery growth. Moreover, the bioreactor is a prototype capable of addressing large-scale production of tissue-engineered arteries.

ACKNOWLEDGEMENTS

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