A Fiber-Reinforced, Large Vessel, Chitosan Scaffold for Pediatric Applications

Irina S. Robu[1], Henry L. Walters III[2], Howard W.T. Matthew[1]
[1]Department of Chemical Engineering & Materials Science, Wayne State University, Detroit, MI
[2]Department of Cardiovascular Surgery, Children's Hospital of Michigan, Detroit, MI

Introduction

Congenital heart and large vessel defects are a significant cause of morbidity and mortality in pediatric patients. Current vessel replacement options lack the capacity to grow and remodel in response to patient needs. This limitation is a major problem for pediatric patients who may require multiple surgical procedures before reaching adulthood. In this study, we explored the prospect of a tissue engineered large vessel graft using polysaccharide biomaterials. Our scaffold design was based on the use of chitosan as the main structural biopolymer, with cell adhesion ligands provided by blending Type I collagen with the chitosan. In addition, we explored the effects of covalently grafting various glycosaminoglycans to the scaffold surfaces to promote binding and presentation of growth factors and extracellular matrix proteins. The aim of the present study is to evaluate a tissue engineered blood vessel in which the extracellular matrix production, collagen and cellular growth can be enhanced and produced similar to natural artery.

Materials and Methods

Scaffold Fabrication

Porous tubular scaffolds with an inner diameter of 12 mm, an outer diameter of 14 mm, and a length of 7 cm were produced by freeze drying a solution of 1.5 wt% chitosan and 0.15 wt% type I collagen in 0.2 M acetic acid within an annular mold containing preformed chitosan fibers. After freeze-drying, scaffolds were rehydrated through an ethanol series and then finally equilibrated in PBS. To improve the cell proliferation and attachment, the scaffolds were then mounted in a perfusion system and surface-derivatized by perfusing carbodiimide-activated heparin through the porous structure. The final heparin immobilization level was 0.2 mg heparin per mg of chitosan. Scaffolds were then washed with PBS and sterilized by immersion in 80 % ethanol for 24 hours. They were then washed with PBS and equilibrated overnight with serum-supplemented culture medium.

Cell Seeding and Scaffold Bioreactor Culture

Porcine aortic smooth muscle cells (SMC) were suspended in 20 ml of culture medium and 30 million cells were seeded into each scaffold by sealing on end of the scaffold and perfusing the cell suspension into the lumen and out through the porous wall. The cell suspension was recirculated for 30 min at a flow rate of 50 ml/min. This resulted in the wall-entrapment of most of the cells. The scaffolds were then placed in 10 cm culture plates and incubated in culture medium for 4 days. The culture medium was MCDB 131 supplemented with amphotericin B, FGF-2, EGF, insulin, 10% FBS and penicillin-streptomycin. During perfusion culture, the medium was additionally supplemented with 0.3 mM ascorbic acid 2-phosphate and 0.1 mM retinoic acid. The seeded scaffolds were then transferred to a perfusion bioreactor system where pulsatile flow through the construct lumen was initiated at a flow rate of 50 ml/min for 4 days. The flow rate was subsequently increased to 100 ml/ min and the culture medium supplementation with 0.1 mM retinoic acid and 0.3 mM ascorbic acid was initiated and maintained for 12 days. Samples were excised from the perfused vessel scaffold at intervals and used to evaluate cell viability and distribution.

Scaffold Analyses

Cell viability was evaluated in scaffold samples using using Calcein red-orange fluorescence.
For scanning electron microscopy (SEM), the scaffold samples were fixed in 2.5 % glutaraldehyde in PBS, dehydrated through an ethanol series, and then flash frozen in liquid nitrogen and lyophilized. After sputter coating with gold samples were examined under an SEM. For histology, scaffolds were

fixed in 10 % buffered formalin, dehydrated and embedded in paraffin. Sections 8-10 μm in thickness were cut and stained with hematoxylin and eosin.

Results and Discussion

Scanning electron microscopy (Figure 1) revealed that the luminal surfaces of heparin-chitosan-collagen after 16 days of growth were covered with smooth muscle cells that were oriented in the direction of the flow.

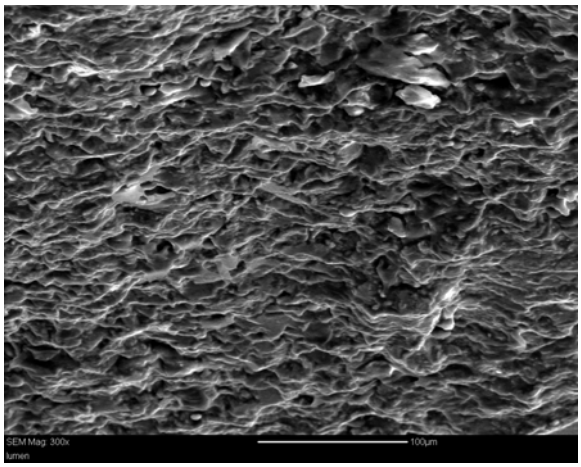
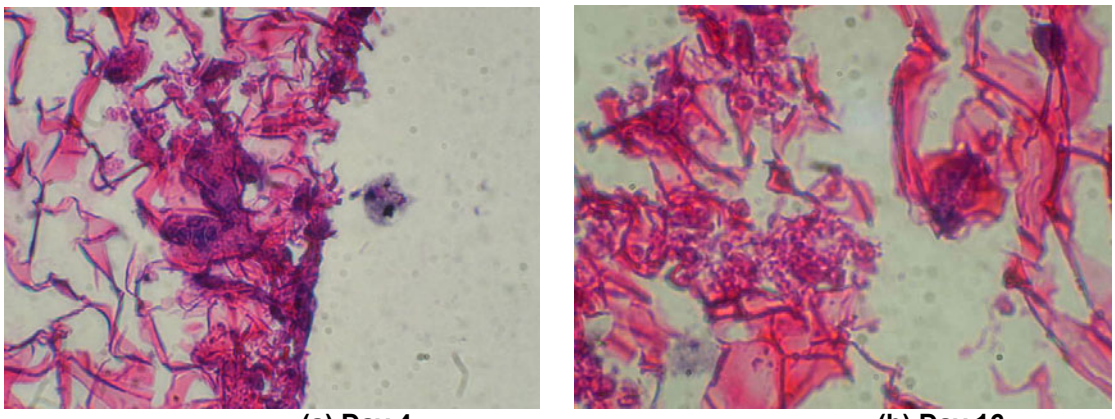


Figure 1. SEM of lumenal surface of heparin-chitosan-collagen scaffolds seeded with porcine aortic smooth muscle cells.

Histology (Figure 2) of the heparin-chitosan-collagen scaffold show clusters of cells present within the scaffold, with the highest cell densities occurring near the scaffold periphery. However, coverage was incomplete and a substantial increase in the amount of cells is needed to fill the scaffold void space.



(a) Day 4 **(b) Day 16**

Figure 2. H & E stained sections of the scaffold on Day 4 of the culture showing SMC near the scaffold periphery

Calcein red-orange fluorescence showed spreading and allignment of SMC. In particular, the cells appeared to align in the direction of flow (Figure 3). shows SMC forming clusters within the scaffold and their viability increased. Figure 3c and 3d shows the cells lining up with the flow and forming arranged clusters. The viability examination of the cells showed that heparin-chitosan-collagen is a viable scaffolding material for engineering large diameter vascular grafts.

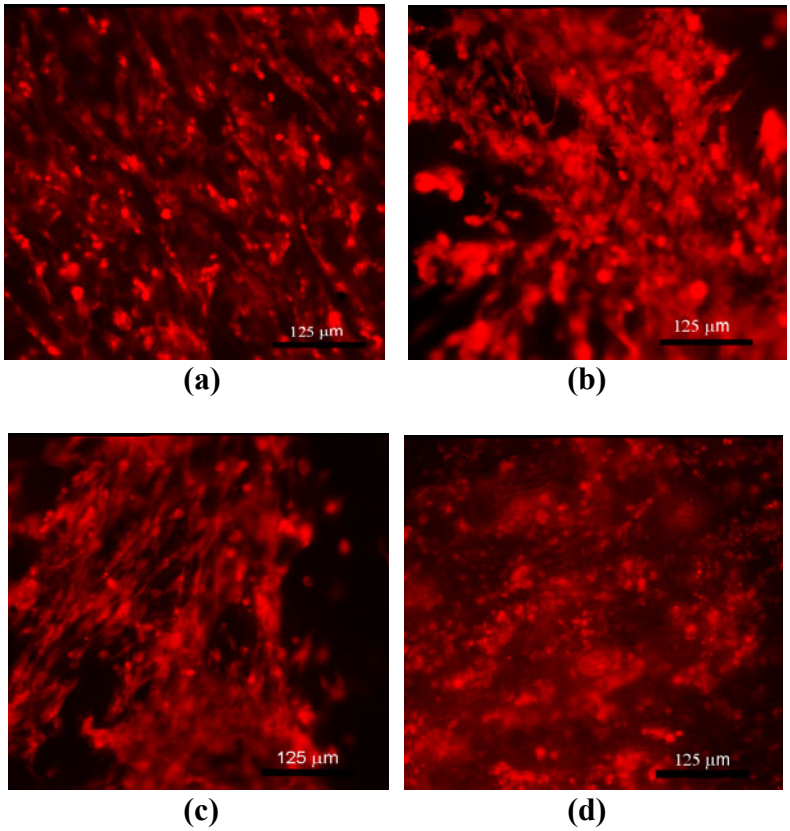


Figure 3. Calcein red-orange fluorescence indicating cell viability scaffold samples taken from the perfusion system on (a) day 2, (b) day 4, (c) day 13 and (d) day 16.

Conclusions

Scanning electron microscopy showed the presence of SMC on the luminal surface, histology examination showed the radial distribution of cells and their penetration within the heparin-chitosan-collagen graft. The graphs were examined regularly for cell viability, and showed high cell viability and aligment of SMC cells in the direction of the flow. We believe that the perfusion seeding produced better cell distribution within the scaffold. However, higher seeding densities are needed to accelerate tissue formation and extracellular matrix deposition.

Acknowledgements

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