Regulation of Material Endothelialization via Polyurethane–Hyaluronic Acid Copolymers

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A. Introduction

Small diameter vascular graft replacement is performed on approximately 600,000 patients in the U.S each year (Dahl SLM, et al. Cell Transplantation 2003;12:659-666). The success of synthetic grafts such as Dacron and Teflon has been limited to large diameter vascular applications (Salacinski HJ et al. J Biomater Appl 2001;15:241-278); in small-diameter applications, these materials experience intimal hyperplasia and thrombus formation. Reasons for graft failure include the inability of these materials to support endothelial cell growth, as well as the tremendous mechanical mismatch between these materials and native vessels (500 vs. 0.5 MPa).

Unfortunately, creation of hemocompatible materials that support endothelialization has been an elusive goal. Hyaluronic acid (HA) is a unique biomolecule that has both non-thrombogenic and angiogenic qualities. Thus, via the copolymerization of polyurethane (PU) with HA, our group has focused on synthesizing materials that are hemocompatible, support endothelialization, and have mechanical properties similar to native vessels (Xu F, Nacker JC, Crone WC, Masters KS. Biomaterials 2008; 29:150-160; Chuang TW, Masters KS. Biomaterials 2009; 30:5341-5351). The current study focuses on tailoring PU-HA materials to enhance endothelialization while retaining vascular-appropriate mechanics, as well as extending this work to 3-dimensional constructs.

B. Methods

Two different HA molecular weights (4.7 and 9.7 kDa) were used as a chain extender in PU synthesis in a range of HA concentrations (0.33-6.0wt% of total reaction). PU and PU-HA porous scaffolds were prepared on glass coverslips by electrospinning a 35-40 g/ml PU or PU-HA polymer solution in (1:1) DMF:$CHCl_3$ or DMF:THF respectively. A 13-17 kV electric field was applied at a distance of 20 cm between the copper plate and the needle tip. Bovine aortic endothelial cells (BAECs) were seeded onto polymer films and porous scaffolds at a density of 20,000 cells/cm^2. Samples were harvested for viability analysis (Live/Dead staining), DNA quantification (PicoGreen), and SEM imaging at time points of 5 hours, and 5 and 8 days post-seeding. The impact of HA amount and MW in PU-HA matrices was also examined with respect to platelet, macrophage, and bacterial adhesion using whole human blood, U937 monocytes, and E. coli, respectively. Young's moduli of PU and PU-HA materials were determined via tensile testing.

C. Results/Discussion

As formation of nanofibrous PU-HA scaffolds has not been previously reported, electrospinning conditions were first designed in order to achieve nanofibrous PU-HA scaffolds (Figure 1). Although PU and copolymer PU-HA materials supported similar initial adhesion of BAECs a significant trend emerged after 5 days, revealing that decreasing HA MW in PU-HA scaffolds was accompanied by an increase in EC proliferation, as quantified by DNA. These trends are also evident in Figure 2, which not only shows almost 100% viability of BAECs on porous PU-HA scaffolds (0.33 wt% HA for both MWs) at 8 days, but also demonstrates how HA molecular weight can affect the extent of scaffold endothelialization.

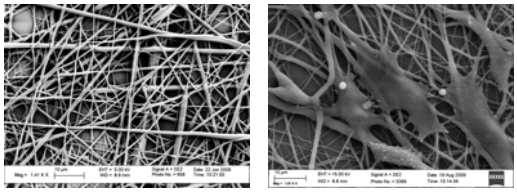

Figure 1: SEM images of electrospun 0.33% PU-HA scaffolds without cells (left) and containing well-spread BAECs (right)

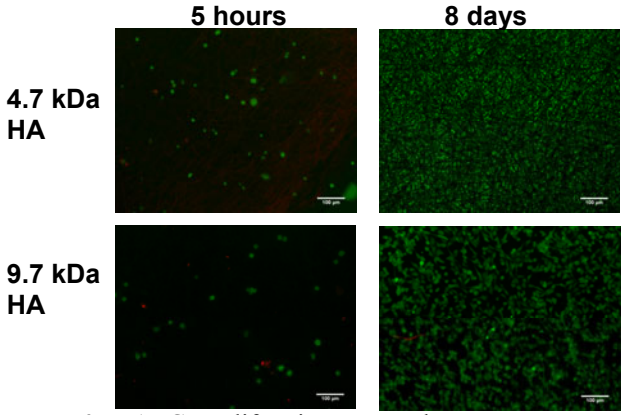

Figure 2: BAEC proliferation upon electrospun porous PU-HA scaffolds varied with the MW of HA in the PU-HA copolymer. At 8 days post-seeding, 4.7 kDa PU-HA porous scaffolds were densely populated with BAECs.

Quantification of platelet, macrophage, and bacterial adhesion on PU-HA materials revealed that adhesion of these cells significantly decreased with increasing HA content, but did not significantly vary with HA MW (not shown). Moreover, mechanical testing confirmed retention of vascular-appropriate mechanics (not shown).

D. Conclusions

Through the incorporation of different molecular weights and amounts of HA, we can control the cellular response to HA-modified materials. Specifically, incorporation of low molecular weight HA into PU enabled the creation of a material that is both non-thrombogenic and supportive of endothelialization. This material has the potential to be used in the creation of small diameter vascular graft.