Regulation of Material Endothelialization and Hemocompatibility via Hyaluronic Acid Grafting

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Introduction

Small diameter vascular graft replacement is performed on approximately 600,000 patients in the U.S each year, constituting one of the most common surgical procedures for treatment of cardiovascular disease (Dahl SLM, Koh J, Ni Niklason LE. Cell Transplantation. 2003;12:659-666). Synthetic materials such as Dacron and Teflon have been developed for vascular grafts, but their success has been limited to large diameter vascular applications (Salacinski HJ, Goldner S, Giudiceandrea A, Edwards A, Hamilton G, Seifalian AM. J Biomater Appl 2001; 15:241-278). When used in small diameter (I.D.≤ 6mm) applications, these grafts fail due to 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 a rather elusive goal in biomaterials research. Hyaluronic acid (HA) is a unique biomolecule in that it has both non-thrombogenic and angiogenic qualities. Thus, via the copolymerization and/or modification 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). As described in this work, changes to the molecular weight of HA used in material synthesis can drastically alter the biological properties of these materials.

Methods

Three different HA molecular weights (4.7, 64, 104 kDa) were grafted onto PU surfaces using carbodiimide chemistry, while heparin (Hep) grafting was performed using sodium periodate-oxidizing chemistry. The mechanical properties of the polymer films were obtained from stress vs. strain curves using an Instron 5548 Micro Tester. Bovine aortic endothelial cells (BAECs, passage 2-11) were seeded onto polymer films at a density of 20,000 cells/cm^2. Samples were harvested at time points of 5 hours and 5 days post-seeding to examine cell number via a dsDNA assay (Quant-iT™ Pico Green®; Invitrogen Corp.). For platelet adhesion studies, coverslips coated with Collagen I, PU, PU-Hep, or PU-HA polymers were incubated for 30 minutes at 37°C followed by five rinses in PBS and fixation in 2.5% glutaraldehyde. The number of adherent platelets was determined by manually counting four randomly-chosen fields of view for each of three samples per condition captured at 200X magnification.

Results/Discussion

As shown in Figure 1, PU, PU-HA and PU-Hep equally supported the initial adhesion of ECs. But, after 5 days of culture, a significant trend emerged, indicating that decreasing HA MW was accompanied by increases in EC proliferation. The number of ECs on the 4.7 kDa HA-modified PU was over 5-fold higher than that found on unmodified PU. Meanwhile, Figure 2 demonstrates that these HA modifications were also successful at significantly inhibiting platelet adhesion ($p<0.001$ vs. collagen or PU controls). The inhibition of platelet adhesion was also significantly better ($p<0.02$) than that achieved using heparin, which has historically been used in attempts to impart hemocompatibility upon biomaterials.

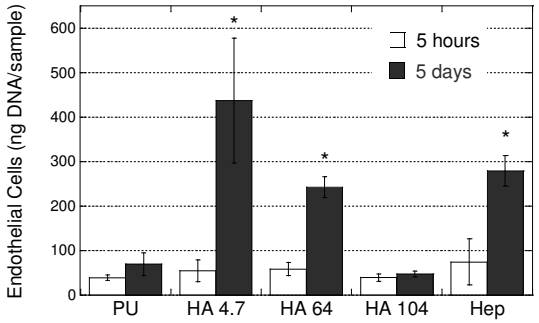

Figure 1: Endothelial cell adhesion/growth on PU, PU-HA, and PU-Hep materials at 5 hours and 5 days. *P<0.05 compared with PU or HA 104 kDa.

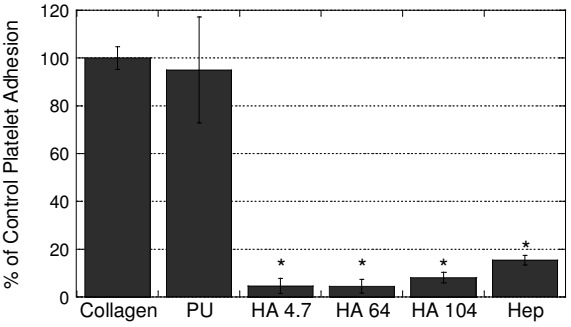

Figure 2: Platelet adhesion was significantly inhibited on all HA- and Hep- modified materials. *P<0.0001 compared with PU or collagen.

Conclusions

Through the incorporation of different molecular weights of HA, we can control the cellular response to HA-modified materials. Specifically, grafting of low molecular weight HA onto PU materials has enabled the creation of a material that is non-thrombogenic, supportive of endothelialization, and has mechanical properties close to that of native vessels.