Photoreactive, cholesterol-containing polymers enhance blood outgrowth endothelial cell attachment to polyurethane

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Statement of Purpose: Endothelialization of the blood-contacting surface of vascular implants would theoretically confer thrombo-resistance. For artificial heart valves, achieving an intact endothelium is made more challenging due to the unique mechanical forces acting upon the valve surface. We have previously demonstrated, both *in vivo* and *in vitro*, that bulk modification of polyurethane (PU) with mercapto-cholesterol significanly enhances blood outgrowth endothelial cell (BOEC) retention under valvular levels of shear force. However, the effect of bulk modification upon the physical properties of PU remains a concern.

We developed a novel surface modification involving a polymeric photo cross-linker (PDT-BzPh) composed of 2-pyridyldithio groups (PDT) linked to the benzophenone (BzPh) photo-reactive groups. We used PDT-BzPh polymers to test the hypothesis that surface immobilized cholesterol will still promote BOEC adhesion and retention under physiological conditions while preserving the mechanical properties of the native PU. The goals of this study were 1. Develop and characterize a surface modification chemistry to covalently append cholesterol to PU surfaces. 2. Assess the effects of this cholesterol modification chemistry with respect to PU mechanical properties and BOEC retention under physiologically relevant shear.

Methods: Ovine BOECs were prepared from heparinized peripheral blood via venipuncture. Mononuclear cells were isolated from blood using Histopaque-1077, collected, resuspended in EGM-2 culture media and seeded onto a tissue culture dish precoated with acetic acid denatured collagen. The culture media was changed every other day and the cells were subcultured using Trypsin/EDTA. Endothelial cell phenotype was confirmed using uptake of acetylated LDL, and immunocytochemistry for both P1H12 and VE-cadherin.

PDT-BzPh was applied to the polyether PU, Angioflex, as a micelle suspension. Under UV-irradiation, BzPH groups form covalent bonds with PU macromolecules. PDT groups, on the PU surfaces, were reduced to Thiol-groups by incubating the films with a solution of TCEP. Two tri-functional water soluble polymers (CPB-1A and CPB-2A) were synthesized containing cholesteryl residues (ca. 20% of all chemical linkages), a carboxylic ionogenic group (65-70% of all linkages), and a thiol reactive group (10-15% of all chemical linkages) composed of either SPDP (CPB-1A) or N-succinimidyl 6-maleimidocaproate (CPB-2A). Some batches of CPB of were modified with the fluorescent probe BODIPY (0.5% of all chemical linkages) to quantify the amount of bound polymer. Mechanical testing examined ultimate stress and strain, tangent moduli, and total work and toughness of control Angioflex and the surface modified CPB-1A and CPB-2A variants. Data was reported as mean ± SD where n=5 samples per group.

BOEC attachment to the modified surfaces was assessed by seeding BOECs onto control Angioflex films, or Angioflex films modified with either CPB-1A or CPB-2A. In some trials, incubating the cells in the presence of the membrane cholesterol disrupter, β1 cyclodextrin, assessed the contribution of cholesterol on BOEC-surface interactions. At the end of the experiments, the films were washed, fixed with 4% paraformaldehyde and assessed for cell attachment via DAPI staining. To analyze BOEC retention under shear conditions, cultured BOECs were seeded on microscope slides coated with control Angioflex, or Angioflex modified with either CPB-1A or CPB-2A, and grown to confluence. Cells were exposed to laminar flow (45 dynes/cm^2) for 2 hours. Slides were washed with saline and retained cells were fixed with 4% paraformaldehyde. Retained cells were stained with DAPI and visualized and quantified using an epifluorescent microscope (200x magnification) and the appropriate filter set. Data was reported as the mean ± S.E.M. n=30 fields counted.

Results: Mechanical testing showed that there were no statistical differences in any measured parameter between unmodified Angioflex, CPB-1A, or CPB-2A films. BODIPY labeling studies determined that the surface concentration of bound cholesterol was approximately 2.0 nM of cholesterol per cm^2, comparable to bulk modified cholesterol PU. In addition, fluoremetry readings showed that the fluorescent label was detected beyond 21 days.

BOEC adhesion studies showed that the presence of surface cholesterol enhanced BOEC attachment 3-fold compared to unmodified Angioflex. These results were statistically significant ($p < 0.001$). The enhanced affinity of BOEC to cholesterol-modified surfaces was reversed with the addition of β1 cyclodextrin. Challenging cultured BOEC with physiologically relevant levels of shear showed that more than 90% of cells seeded on CPB-1A and CPB-2A were retained following two hours exposure to high levels of shear. In contrast, only 5% of BOEC, seeded on control Angioflex surfaces, were retained.

Conclusions: We conclude that modification of PU surfaces, with cholesterol, has no untoward effects upon the polymer's mechanical properties. In addition, surface application of cholesterol provides a high affinity surface for the attachment and retention of BOEC. Modification of polyurethane heart valves, via photoactivation chemistry, with cholesterol is a plausible endothelialization strategy that will not compromise the mechanical properties of the artificial valve.