# HDL-Adsorbing Hydrophilic Surfaces: Potential as Blood-Compatible Endothelium Regenerating Coatings.

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**Introduction.** Synthetic small-diameter vascular prostheses frequently fail because of thrombotic complications, inflammation, or neo-intimal hyperplasia.[1] The underlying problem is that the luminal surface lacks the potential to support regeneration of the vascular endothelial cell (EC) layer. Normally the endothelium ensures proper regulation of blood coagulation and prevents inappropriate binding and activation of leukocytes. Therefore an intact and functional layer of endothelial cells should be achieved on the implant surface. In addition to this, the interaction with ECs should be specific. This means that interaction with vascular smooth muscle cells (VSMC), leukocytes or platelets should be absent. Endothelialization of synthetic surfaces has proven difficult to achieve in animal models and human patients. Often surfaces that favor the adhesion and growth of endothelial cells, e.g. collagen and RGD-exposing surfaces are not specific and also bind platelets or leukocytes or VSMCs. This may result in thrombotic or inflammatory complications and failure of the synthetic vascular prosthesis.
In an earlier study we have demonstrated that polymeric hydrophilic coatings, with adsorbed high-density-lipoprotein (HDL), strongly enhance endothelialization.[2] Here we investigated the specificity of endothelial cell adhesion to the hydrogel-HDL surface. In addition the effect of vascular cell or leukocyte adhesion on surface induced thrombin generation is reported.

**Methods.** The hydrophilic copolymer, consisting of 90 mol% N-vinyl-pyrrolidinone (NVP) and 10 mol% n-butylmethacrylate (BMA), was uniformly applied onto a thin metallic wire.[3] HDL, Apo-A1 (the main protein component of HDL) or a synthetic form of HDL were adsorbed to these wires. As a control, wires with a hydrophobic coating (NVP/BMA 10/90) were used, since HDL will not adsorb to this coating. Alternatively, HDL was covalently coupled to microspheres that contain 10 mol% 2-propenal (acrolein) under mild conditions.[4]
Adhesion and proliferation of endothelial cells, VSMCs, and/or fibroblasts was determined. A mixture of ECs and VSMCs or fibroblasts was used to determine specificity of ECs for the hydrogel-HDL surface. Also adhesion and activation of platelets and leukocytes was studied. Fir this, platelet-rich-plasma (PRP) or leukocytes were isolated from fresh human peripheral blood. Leukocytes were resuspended in plasma and incubated with surfaces with or without HDL. Platelet adhesion and activation was determined using PRP. Finally, the generation of thrombin on surfaces with attached cells or leukocytes was measured using a static fluorescence-based assay.[5]

**Results.** Hydrogel-HDL surfaces supported the adhesion and growth of endothelial cells, but not that of VSMCs or fibroblasts (Figure 1). On surfaces without HDL or surfaces with low-density-lipoprotein (LDL), no cell adhesion and growth were observed.

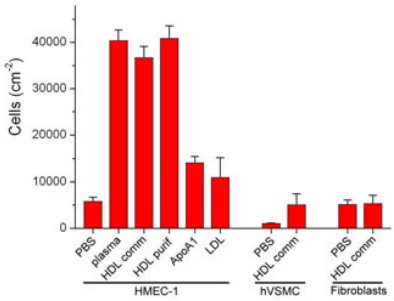


Figure 1. Adhesion and growth of endothelial (EC), smooth muscle (VSMC) cells, and fibroblasts on hydrophilic surfaces treated with HDL.

Incubation of surfaces with adsorbed or covalently linked HDL showed strongly reduced adhesion of platelets and leukocytes. Also activation of platelets and leukocytes was inhibited. Thrombin generation was stimulated by surface-adhered leukocytes. However, HDL reduced thrombin generation and thus thrombus formation on such blood-cell primed surface (Figure 2).

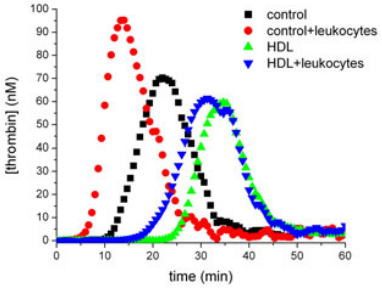


Figure 2. Thrombin generation on surfaces with adhered leukocytes. The hydrogel-HDL coatings show a reduced and delayed thrombin generation.

**Conclusions.** Hydrophilic coatings with adsorbed or covalently coupled HDL are promising as blood-contacting surfaces. These coatings specifically stimulate regeneration of endothelium. Additionally, HDL improves blood-compatibility by inhibition of platelet and leukocyte adhesion and activation.

**References:**

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