Modification of Polymeric Surfaces with High Density Lipoprotein Strongly Improves Blood- and Cell-Compatibility.

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Introduction

Blood-contacting devices, like vascular prostheses and stents, often fail because of thrombotic complications and/or intimal hyperplasia. The surface of these devices initiate a sequence of events that starts with the adsorption of plasma proteins and results in activation of platelets and leukocytes, thrombin generation, and ultimately formation of a blood clot. Incorporation of anti-coagulant drugs, like heparin, in surface coatings has lead to improved blood-compatibility.[1] Also, modification with protein-repellent poly-ethylene-oxide, has resulted in improved thrombo-resistance.[2] Despite these developments, it has become apparent that application of such synthetic surfaces does not lead to acceptable long-term blood-compatibility. Many of the problems associated with permanent blood-contacting devices are caused by the failure of the surface to induce and support a functional endothelial cell layer.

In earlier studies we described that adsorption of high-density-lipoprotein (HDL) to hydrophilic polymeric coatings results in drastic improvement of surface endothelialization. Also, thrombin generation on these surfaces was strongly inhibited.[3] We therefore set out to extend our studies on blood-contacting surfaces modified with HDL. For this we developed polymers that can be covalently modified with HDL. Additionally we studied the mechanisms by which HDL enhances endothelialization and improves blood-compatibility.

Methods

A copolymer of N-vinyl-pyrrolidone (NVP) and butyl-methacrylate (BMA), molar ratio 9:1, was applied to thin metallic wires, which were subsequently wound into a flexible coil.[1] Purified HDL, HDL from human plasma, and synthetic HDL preparations were specifically adsorbed onto these coils.[3] Polymeric microspheres composed of methyl-methacrylate (MMA) and acrolein (2-propenal) were used to study the effect of covalently linked HDL. Coupling of HDL was performed under mild basic conditions followed by reduction of the formed Schiff base. The HDL modified surfaces were studied for: 1) adhesion and proliferation of human microvascular endothelial cells (HMEC-1), 2) adhesion and activation of platelets and leukocytes, 3) the generation of thrombin under static and flow conditions in platelet-rich-plasma and whole blood. Adsorption or coupling of low-density-lipoprotein (LDL) was used as control.

Results and Discussion

Modification of polymeric surfaces with HDL results in a strong stimulation of endothelialization. More detailed studies demonstrate that HDL does not increase the rate of proliferation of the endothelial cells, but strongly improved the adhesion of these cells.

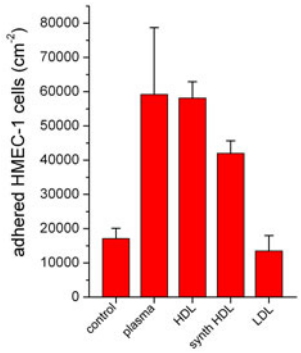

Figure 1. Adhesion of HMEC-1 cells to hydrophilic polymeric coating modified with human plasma, HDL, synthetic HDL and LDL.

Furthermore, HDL modification of polymeric surfaces resulted in a strong decrease of platelet and leukocyte adhesion. The generation of thrombin and subsequent thrombus formation was strongly slowed down on surfaces with adsorbed or covalently bound HDL

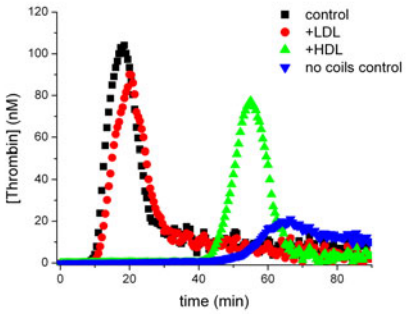

Figure 2. Polymeric microspheres with covalently linked HDL demonstrate inhibition of thrombin generation under static conditions. Modification with low-density-lipoprotein (LDL) is used as control.

Conclusions

Modification of synthetic polymeric surfaces with HDL results in improved endothelialization and blood-compatibility. The adhesion of endothelial cells is strongly enhanced by HDL.

References:

1) Aldenhoff, YBJ. J Vasc Interv Radiol 2007;18:419-425
2) Zhang Z. Biomaterials 2008;29:4285-4291.
3) Knetsch MLW. Biomaterials 2006;27:2813-2819