High Density Lipoprotein enhances Endothelialization of Synthetic Surfaces *in vitro* and *in vivo*.

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Introduction

Synthetic small diameter vascular prostheses fail frequently because of thrombotic complications or inflammation.[1] The underlying problem is the lack of endothelialization of the blood contacting surface. Only a functional endothelial cell layer can ensure efficient haemostatis control and prevent inflammation. In situ endothelialization 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 thrombogenic which can eventually lead to failure of the vascular graft.

In previous studies we have demonstrated that hydrophilic blood-contacting surfaces, which specifically adsorb high-density-lipoprotein (HDL), strongly enhance spontaneous endothelialization *in vitro*.[2] Additionally, HDL improves blood-compatibility of the surface by inhibiting the generation of thrombin and reducing platelet and leukocyte adhesion.[2] In this study we assess the *in vivo* endothelialization of our synthetic vascular grafts, coated with a hydrophilic polymer and adsorbed HDL. Furthermore, the hydrophilic surface is covalently modified with HDL to prevent its release post-implantation. The hydrogel-HDL surface is assessed for endothelialization and blood-compatibility.

Methods

The hydrophilic polymer, consisting of 90 mol% N-vinyl-pyrrolidinone (NVP) and 10 mol% n-butylmethacrylate (BMA), was uniformly applied onto a thin metallic wire.[3] The coated wire was coiled to obtain a vascular prosthesis with uniformly coated luminal surface.[3] Grafts with an inner diameter of 2.5 mm and length of 5 cm were implanted as interposition grafts into the carotid arteries of goats. As a comparison, vascular prosthesis with a hydrophobic coating of NVP/BMA 10/90 was used. HDL will not adsorb to this coating.

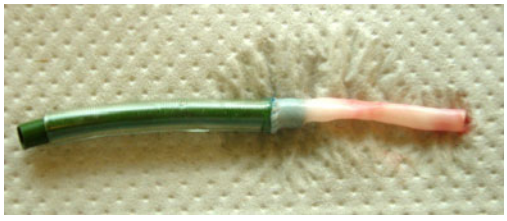

Figure 1. Prototype of vascular prosthesis attached to a dissected piece of goat carotid artery.

The grafts were explanted after 3 months, and the endothelial cell formation on the surface was assessed by scanning electron microscopy (SEM) and histology.

To study the effect of covalent coupled HDL, polymeric microspheres were synthesized that contain 10 mol% acrolein (2-propenal). HDL was coupled under mild conditions to these spheres at pH 9.0 in 50 mM carbonate buffer.[3] The *in vitro* adhesion and growth of EC cells was studied. Also, *in vitro* generation of microsphere induced thrombin in platelet-rich-plasma was determined.

Results and Discussion

The implantation of the prototype vascular prostheses was straightforward and posed no problems. After 3 months, the vascular grafts were explanted, and found not to be occluded. Careful observation of the luminal surface demonstrated a cell layer with typical cobblestone morphology (fig 2).

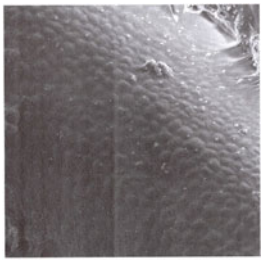

Figure 2. SEM image of the luminal surface of the vascular prosthesis after explantation.

Covalent attachment of HDL onto polymeric microspheres resulted in increased endothelial cell adhesion and growth. Finally, the generation of thrombin on surfaces with covalently coupled HDL was strongly inhibited, very similar to adsorbed HDL. This means that covalently coupled HDL performs as efficient as the adsorbed HDL.[2]

Conclusions

Hydrophilic coatings with adsorbed or covalently coupled HDL are promising as blood-contacting surfaces for synthetic small diameter vascular prosthesis. These coatings enhance endothelial cell adhesion and consequently endothelialization of the vascular prosthesis. Additionally, covalently coupled HDL improves blood- and cyto-compatibility. Therefore, the hydrogel-HDL coating may also be a candidate for stent-coating since it induces *in situ* coverage with an endothelial cell layer.

References:

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