Development of new toxin-adsorbing and hemocompatible surfaces as a step towards a miniaturized artificial kidney

Jorrit H. Janssen and Leo H. Koole
Maastricht University, Department of Biomedical Engineering/Biomaterials Science, Maastricht, the Netherlands.
INterface BIOmaterials BV, B. Lemmensstraat 364, 6163 JT Geleen, the Netherlands

Statement of Purpose: More than 1 million people worldwide suffer from end-stage renal disease (ESRD). Hemodialysis is an effective treatment for ESRD patients, but it has a heavy impact on the quality of life [1]. Improved techniques for clearance of toxins from a patient's blood are being developed. We study a technique to specifically adsorb protein-bound toxins; it is well known that such toxins are not removed effectively by dialysis [2]. When combined with routine dialysis, the adsorption method could improve the procedure. A supplementary small unit containing the adsorption material can be sufficient to remove protein-bound toxins. The surface of such material used for an adsorption device should meet the requirements to a) come in contact with blood to adsorb toxins, b) be specific to adsorb toxins only, c) have a blood-contacting surface that is as large as possible, d) have a stable coating (no leakage of coating) and e) be hemocompatible.

Methods: Our approach is based on the SlipSkin™ copolymer with the composition 30/70 (manufactured from NVP and n-BMA in the mass : mass ratio 30 : 70). This biomaterial was tested by an external certified lab following internationally accepted guidelines and recommendation for biological evaluation of medical devices. The biomaterial was found to be non-cytotoxic (according to DIN EN ISO 10993-5 ("Tests for in vitro cytotoxicity")) and hemocompatible (according to DIN EN ISO 10993-4 ("Selection of tests for interactions with blood"). Sodium heparin (175 USP units/mg) and carbon micro particles were mixed with the coating solution, and the mixture was stirred to obtain a homogeneous suspension. This suspension was used for the extrusion like coating procedure [3]. The coating was applied to a thin stainless steel wire (Ø=78 µm). In this way a very large surface/volume ratio was obtained. Indoxyl sulfate concentrations were quantified through XPS analysis with a Quantera SXM™.

The coated wire was coiled, and the coils were put in a tube containing spiked toxin amounts of creatinine and/or indoxyl sulfate. Samples were taken regularly (i.e. hours or days) and toxin concentrations were determined. Note that indoxyl sulfate is a model for protein-bound toxins.

Hemocompatibility experiments were performed with fresh human blood, obtained via venipuncture from healthy donors who have not taken aspirin or any other anticoagulant one day prior to blood donation. Thrombin generation experiments were performed using the fluorescent substrate Z-Gly-Gly-Arg-AMC. This substrate cleaves in the presence of thrombin, and fluorescence of the released AMC is measurable at 365 nm. The coated material was subjected for 60 minutes in platelet rich plasma (PRP) and every 30 seconds the thrombin concentration was evaluated. At higher concentrations of thrombin, the enzyme effectively cleaves fibrinogen to generate fibrin, which is the actual start of clotting (thrombin generation time or TGT). Platelet adhesion was studied in PRP via the measurement of lactate dehydrogenase (LDH), a stable cytosolic enzyme that is released upon cell lysis.

Results: The samples taken at regular time points during the incubation of the coated wire in the toxin solution were analyzed for creatinine and indoxyl sulfate concentrations. The longer the coated wire was incubated, the less toxin concentration was present in the fluid, which means that the toxin was adsorbed in the time by the coating's surface. The coated wire adsorbed creatinine for almost one year (saturation point is not yet determined). Similar adsorption behavior was also found for the protein-bound toxin indoxyl sulfate. The hemocompatibility assays were performed on SlipSkin™, carbon and heparin coatings and its other possible combinations. The SlipSkin™/carbon/heparin coating was less thrombogenic (higher TGT) than SlipSkin™ alone (Figure 1).

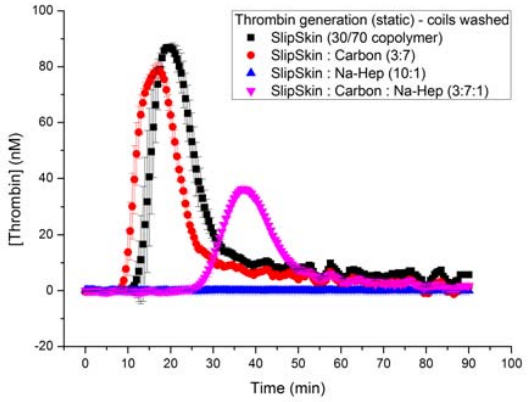

Figure 1. Thrombin generation curves for SlipSkin™, impregnated with heparin or carbon, or both.

Conclusions The SlipSkin™ surface coating, with sodium heparin and carbon micro particles embedded therein, adsorbs the toxins creatinine and indoxyl sulfate, while the thrombogenicity remains low. This finding, may be relevant with regard to the development of miniaturized artificial kidneys.

References:

1. Davenport A. Lancet 2007;370:2005-2010
2. Lekawanvijit S. Eur Heart J 2010;31:1771-1779
3. Aldenhoff YBJ. Biomaterials 2004;25:3125-3133