

## CARRIERS FOR EXTRACORPOREAL BLOOD PURIFICATION: FROM BASIC RESEARCH TO HEALTH CARE

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The concept of removing pathogenic agents from the body by blood purification procedures is certainly one of the oldest therapeutic approaches in the history of medicine starting in ancient time with therapeutic bloodletting.

Traditional ways for the removal of targeted substances from the blood by means of extracorporeal devices are centrifugation, membrane permeation such as hemodialysis and hemofiltration and adsorption.

The extracorporeal centrifugation system has certain limitation to separate among macromolecule, needs appropriate and physical alteration to produce aggregates, is technical complicated and very costly.

Due to modern membrane and electronic technology dialysis and filtration are biocompatible, efficient and safe. On the other hand the membranes used in those procedures do not enable the possibility of the specific removal of substances. As an example in hemodialysis or even in hemofiltration the elimination of mostly hydrophilic and not or only weakly protein-bound substances is based on diffusive or convective transport due to semiconductive and porous properties of the membrane. Dialysis treatment started 60 years before and is now a well-established procedure for the removal of uremic toxins having a relative molecular mass below 12,000.

Plasmapheresis or continuous plasma exchange was developed with the invention of cell separators and plasma filters in the early 1970s. A large number of meanwhile published reports suggest that plasma exchange has been performed in desperate cases of almost every systemic disease. Controlled clinical studies, however, are rare, although some indications are supported by a substantial body of evidence. Plasma exchange can be very efficient in acutely removing pathogenic plasma constituents until a relative molecular mass of 2 to 2,500,000, such as autoantibodies (AAB) and circulating immune complexes (CIC), if a sufficient blood volume is processed. One has to remember, however, that clinical relevant side effects especially upon substitution with human plasma protein solutions are frequent. Although of minor relevance in cases of life threatening

disease, the risk of transmission of infectious diseases has also to be taken into account.

Treatment using plasmapheresis or modified procedures in combination with adsorption based on modified microspheres is very useful for the removal of albumin bound toxins in patients with liver diseases [1-3]. So far young companies have developed successful liver support systems in order to use combined membrane-adsorption systems as successful liver support systems especially for bridging to transplantation.

The specific or even selective removal of substances of different relative molecular mass requires the use of functionalized surface either the membrane and their pores or of adsorbent particles. Adsorption processes are based on different binding forces such as hydrophobic interaction due to van der Waal's forces, hydrogen, electrostatic or covalent binding. Nevertheless the surface area is almost of highest importance. For example, 1  $\mu\text{m}$  particles in a volume of the space of 1 mm particle have a 1000 times larger surface.

But more substantial is the inner surface, which is very characteristic for substances such as activated charcoal, resins or other polymers. The total surface area can reach 2000  $\text{m}^2/\text{g}$ . The amount of 50-100 g of particles with such a surface is enough to remove the pathological toxins e.g. bilirubin from one patient. The advantage of the smaller particle with pores in comparison with normally used 100-300  $\mu\text{m}$  porous particle is the much shorter diffusion time, as demonstrated for different adsorbents and target molecules.

Especially adsorbent technologies based on functionalized surfaces using immobilized antibodies or specific peptides, spacer linked aromatic groups for the removal of IgG or even cationic groups such as DEAE (diethyl-aminoethyl) - groups for the removal of endotoxins are very efficient in case of using microparticle technology. Of course, in case of highly porous adsorbents there is no difference of the adsorption capacity comparing to the same volume of adsorbent of the given diameter. But for many substances investigated the adsorption could be reached after hours using particle in the range of 200  $\mu\text{m}$  in comparison to the microparticles of 1-5  $\mu\text{m}$ . Their adsorption capacity normally is reached after minutes at a given concentration.

Extracorporeal immunoadsorption (IA) is a form of plasmapheresis based on affinity chromatography [2,3] with particles sized 100 – 250 µm.

In comparison to plasma exchange, IA allows more or less selective elimination of pathogenic substances from plasma without disposal of valuable plasma proteins. The profile of adsorbed plasma constituents depends on the nature of interaction with the respective ligand. A number of systems are approved and available on the market. For instance CIC and AAB can be eliminated by columns with either tryptophan or phenylalanine immobilized to a polyvinyl ethanol gel [4]. These columns have been used successfully in the treatment of SLE patients eliminating anti-dsDNA antibodies and CIC. Considerable data from clinical queries have been also accumulated for protein A coupled to Sepharose (Immunosorba, Excorim AB, Lund, Sweden) or to silica (Prosorba, Fresenius HemoCare / Cypress Bioscience Inc., San Diego, California) [5]. The affinity of protein A columns to CIC is much higher than to single IgG molecules because of IgG cluster formation in CIC.

The removal of AAB and CIC from the circulation has been postulated as primary mechanism of action of IA. Newer clinical studies, however, show beneficial effects of IA in disorders that appear to be mainly T-cell-mediated. One might place IA into the new context of being a modulator of immune response.

The patient-specific immunoadsorber (psIA) is capable to selectively remove CIC. The adsorber filling consists of a carrier material derivatized with a protein A-purified patient plasma fraction as ligand. The individualized column appears to have binding properties selective for plasma constituents of the individual patient. The choice of a suitable carrier material, optimization of the activation method (ONB-activation; ONB: 5-norbornene-2,3-dicarboximido carbonochloridate) and immobilization of dissociated antigen/antibody-complexes on activated support were important milestones in the development of the psIA. They were underlined by studies of immune complex models like human IgG/anti-human IgG, human serum albumin/anti-human serum albumin, and human transferrin/anti-human transferrin. Analytical data from in vitro experiments carried out in parallel by FPLC and batch procedures show surprising elimination characteristics.

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