New insights in dialysis membrane biocompatibility: relevance of adsorption properties and heparin binding

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Introduction

In the last 10 years, the concept of biocompatibility as applied to haemodialysis membranes has gained a general clinical acceptance in light of the knowledge that the clinical expression of β₂-microglobulin (β₂m) amyloidosis found in long-term haemodialyzed patients was governed by the characteristics of dialysis membranes. Highly permeable and biocompatible synthetic membranes interfere with the clinical expression of the disease and postpone occurrence of carpal tunnel syndrome and juxta-articular bone cysts [1–4].

Biocompatibility is a difficult concept to define in the absence of well-established clinical correlates. Besides ‘anaphylactoid reactions’ [5], which are related more frequently to contaminated dialysate or leachable chemicals than to the sustained activation of the contact phase of coagulation in patients treated with angiotensin converting enzyme inhibitors [6,7], the concept of biocompatibility refers to any harmful effects induced by the contact of blood with dialysis membrane [8]. We consider that a membrane is less biocompatible when the sum of adverse humoral and cellular reactions occurring during haemodialysis is higher than for a reference membrane. An extended definition of biocompatibility should also include factors related to the patient’s clinical conditions, such as diabetes or systemic inflammatory disease, and to factors associated with the specific supportive technique: i.e. haemodialysis, haemofiltration, haemodiafiltration, acetate-free dialysis, etc.

The aim of this short review is to highlight the key roles played by the adsorptive properties of dialysis
membranes in determining biocompatibility and to extend the advantages of this property to heparin binding.

Membrane components and effect of extracorporeal devices

A wide spectrum of cellulosic and synthetic materials has been used for manufacturing dialysis membranes. Some of the synthetic membranes in wide use are made with one of the following polymers (this list is not exhaustive): polysulfone, polyamide, polyacrylonitrile, and their copolymers, polymethylmetacrylate, polytetrafluoroethylene and their various derivatives. It may be pointed out that some of these products were developed primarily for industrial use and later found their way in to medical devices.

The use of high-flux dialyzers lead to understanding the role of uraemic toxins [9–11] and diffusion of endotoxin from contaminated dialysate by backfiltration, and to the general acceptance of the hypothesis of ‘membrane bioreactor’ proposed by Henderson et al. [12]. It suggested, in short, that monocyte activation induced by endotoxin stimulated synthesis and secretion of the proinflammatory interleukin-1 (IL-1).

Furthermore, it has been documented that other proinflammatory and chemoattractant cytokines such as TNF-α, IL-6 and IL-12 [13,14] are secreted in excess in haemodialysis patients and, to return to biocompatibility, it was demonstrated that generation of pro-inflammatory cytokines was also induced by the direct contact of leukocytes with cellulosic membranes in the absence of endotoxin [15–17]. Studies of plasma levels of cytokines have provided conflicting clinical results about their values as prognostic markers in the treatment of acute renal failure. Nevertheless, the use of synthetic highly permeable membranes, which bind endotoxins, cytokines and growth factors in conjunction with high purity dialysates (the lowest possible endotoxin concentration as measured by up-regulation of the endotoxin receptor CD14 at the surface of circulating leukocytes), is recommended by the European Best Practice Guidelines [18]. The binding properties of membranes have made possible the practical application of haemodiafiltration with one-line products of substitution fluid administered intravenously without prior control for biological qualities.

Dialysis membranes can be classified according to their permeability to plasma proteins. However, that classification does not take into account their binding capacities, which differ from one membrane to the other and govern, at least partly, the clearance of charged molecules. Membrane biocompatibility, which is mainly associated with the binding characteristics of polymers, is related to the distribution of hydrophobic and hydrophilic domains and electric charges at the surface and in the body of the membrane. A classification can be proposed based on measurements of the Zeta potential (Table 1) [19].

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Zeta potential (mV)</th>
<th>Plasma kallikrein (U/l)</th>
<th>Bradykinin generation (fmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AN69</td>
<td>−70 ± 5</td>
<td>60 ± 15</td>
<td>52 100 (26 500–41 200)</td>
</tr>
<tr>
<td>PANDX</td>
<td>−60 ± 4</td>
<td>80 ± 20</td>
<td>8983 (22 600–36 150)</td>
</tr>
<tr>
<td>PMMA</td>
<td>−25 ± 2</td>
<td>10 ± 5</td>
<td>130 (50–250)</td>
</tr>
<tr>
<td>CT</td>
<td>−20 ± 2</td>
<td>&lt; 5</td>
<td>65 (25–100)</td>
</tr>
<tr>
<td>CUP</td>
<td>−10 ± 1</td>
<td>&lt; 5</td>
<td>78 (25–50)</td>
</tr>
<tr>
<td>PS</td>
<td>−51 ± 4</td>
<td>&lt; 5</td>
<td>62 (25–120)</td>
</tr>
<tr>
<td>AN69-ST</td>
<td>−15 ± 4</td>
<td>&lt; 5</td>
<td>150 (30–450)</td>
</tr>
</tbody>
</table>

Membranes: polyacrylonitrile AN69 (Hospal); PANDX: polyacrylonitrile (Asahi); PMMA: polymethylmetacrylate (Toray); CT, cellulose triacetate (Baxter); CUP, cuprophane (Akzo); PS, polysulfone (Fresenius); AN69-ST, AN69-PEI (Hospal).

Physicochemical mechanisms associated with membrane biocompatibility

In clinical haemodialysis, the concept of biocompatibility was first coined after the discovery that the dramatic drop of circulating leukocytes noted during a session of haemodialysis performed with a cellulosic membrane did not occur when using the synthetic membranes [20,21]. This effect was the result of complement activation that generated anaphylatoxins, and of the up-regulation of the surface adhesion molecules and the expression of leukocyte antigens, such as CD11b, CD18, complement receptor type 3 and CD45 [22–25]. Biomaterial is recognized by the complement system as foreign and triggers its activation. When complement activation is initiated, biologically highly active inflammatory mediators are generated, including the anaphylatoxins C3a, C4a and C5a, the opsonin iC3b and the terminal complement complex C5a exerts a particularly strong proinflammatory activity by inducing an up-regulation of granulocytes, monocytes, lymphocytes and platelet adhesion molecules and lysosomal release from polymorphonuclear granulocytes. It is generally accepted that synthetic membranes are less harmful to complement, leukocytes and platelets than cuprophane, because they up-regulate adhesion to a lesser extent and, in addition, some of them have the unique property for binding anaphylatoxins, cytokines and enzymes released by cell activation after membrane contact [17,26,27].

The activation of polymorphonuclears by artificial membranes induces a burst of oxygen-free radicals, the so-called ‘oxidative stress’ and associated with it the ‘inflammatory stress’ that together induce strong biochemical transformations of lipids and proteins (peroxydation) and generate toxic radicals that activate endothelial cells and result in vascular injury and atherosclerosis. It is now well documented that chronic inflammation, as indicated by high plasma levels of C-reactive protein, can be generated by either of two mechanisms: blood contamination by bacterial endotoxin originating from contaminated dialysate and diffusing into the blood, and by the simple
contact of IL-1-generating cells such as monocytes with incompatible membranes [23].

Criteria for measuring dialysis membrane biocompatibility are numerous. Many of them were developed in vitro, and some discrepancies have been reported when they were applied in vivo. We do not know to what extent the disturbances induced by the contact between blood and artificial membrane trigger general defence mechanisms, as we are unable to measure all the buffering systems that support our defences against inflammatory stress. Nevertheless, checking these pathophysiological mechanisms is mandatory in order to avoid or reduce the reactions associated with subacute inflammation—accelerated cardiovascular disease and malnutrition—that characterize patients on chronic haemodialysis.

**Haemocompatibility of dialysis membranes**

Haemocompatibility is an aspect of dialysis biocompatibility, one that has been particularly well studied in the context of extracorporeal circulation for cardiopulmonary surgery. In the absence of anticoagulants, all membranes induce the contact phase activation and subsequent reactions of blood clotting [28–31]. Blood coagulation results from the conversion of fibrinogen into insoluble fibrin. The fibrin is deposited on the membrane surface and forms a mesh of fibrils. In contact with fibrin, platelets are activated and aggregate, giving rise to a cooperative interaction that leads to blood clotting. Simultaneously, blood cells are attracted by the thrombus, invade it and, through enzymatic release, contribute to further fibrin formation and platelet recruitment. These series of reactions and binding of clotting molecules onto the membrane surface contribute to the formation of a biological membrane that spread over the polymer and is conventionally called ‘protein cake’. This biological layer contains plasma proteins such as Factor XII, fibrinogen, vitronectin, high molecular weight kininogens (HMWK) and others whose activation results in further thrombogenesis [32–35], strong platelet aggregation and release of procoagulants like platelet Factor 4. We have seen that leukocytes also are involved in this process and by secreting proteases, lactoferrin and myeloperoxidase contribute to the proinflammatory reactor of membrane incompatibility [26].

**Dialysis membrane adsorption**

Exposure of blood to artificial membrane surfaces almost immediately leads to the adsorption of plasma proteins. This initial adsorption profoundly influences all subsequent events, and to a large extent, determines the thrombogenicity, in other words the biocompatibility of the material. It has been proposed that there are two steps of plasma protein membrane adsorption. The first occurs on the membrane surface as a result of preferential competitive adsorption of high molecular weight proteins, such as albumin, fibrinogen/fibrin, fibronectin and globulins. This phenomenon has both a limited capacity and a limited selectivity due to the saturation of the surface with albumin and clotting proteins. The second step of adsorption, which follows surface adsorption, is absorption in the body of the membrane of proteins with low and medium molecular weights. This phenomenon is slower, depends on membrane structure and thickness and is limited by membrane permselectivity. It involves proteins like β2m, cytokines and anaphylatoxins. This process is dynamic, with continuing enzymatic reactions and substrate replacement. However, its overall turnover rate has not been measured in vivo. A technique using radiolabelled proteins and at the end of a session measuring the radioactivity trapped in the dialyzer has yielded some data for the capacity of dialyzers to bind β2m [36].

Adsorption on the AN69 membrane of HMWK, which participates in the contact phase activation of the endogenous blood coagulation cascade has been demonstrated in vitro [37–39]. The membranes biological activity was maintained and correlated with the amount of adsorbed protein. The mechanism of HMWK adsorption was dependent on ionic interactions between the membrane and the protein. Neutralization of charged surface anionic sulfonate groups scattered over the polymer by polyethylene-imine (PEI), as in the AN69-ST membrane, significantly decreased HMWK binding and decreased kinin generation. PEI reduced surface electronegativity without altering the negatively charged sulfonates of the membrane core. The increased biocompatibility of this new membrane was also credited for improving the binding of C3a, C5a [16], Factor D [27] and glycated β2m [40].

The protein layer coating the polymer reduces membrane permeability throughout a dialysis session [41]. This event results from the plasma proteins and adherence of platelets and leukocytes onto haemodialysis membranes. It depends on a number of factors, among them: the nature and the thickness of polymer; the kind of plasma proteins; the interaction between membrane and protein electric charges and local pH; the type of anticoagulant in use; the flow properties of the system. Tailoring the physico-chemical properties of the polymer smooth surface to selectively adsorb a protein layer to include an anticoagulant such as heparin, or an enzyme such as uricase or an antioxidant such as vitamin E has been proposed as a means of improving haemodialysis [42]. The degree to which a particular binding occurs depends to a large extent, on the character of the membrane—whether it is porous or non-porous, hydrophobic or hydrophilic, polar or non-polar. The occurrence of so many interactions raises the question of competitive adsorption of proteins and renders the design of membranes with specific sorbent properties more difficult. Table 2 shows changes of protein adsorption induced through the neutralization of surface electronegativity by covering the AN69 surface with PEI.
Table 2. Mean value of protein adsorption onto native and modified AN69 membrane

<table>
<thead>
<tr>
<th>Plasma proteins</th>
<th>Molecular weight (Da)</th>
<th>Isoelectric point</th>
<th>Adsorption at pH 7.4 (mg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>AN69</td>
</tr>
<tr>
<td>HMWK</td>
<td>120 000</td>
<td>4.45–4.65</td>
<td>1.5</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>340 000</td>
<td>5.10–6.3</td>
<td>30</td>
</tr>
<tr>
<td>β₂-m</td>
<td>11 800</td>
<td>5.7</td>
<td>36</td>
</tr>
</tbody>
</table>

Reducing surface electronegativity by PEI (AN69-ST membrane) significantly decreased HMWK and fibrinogen binding, whereas binding of β₂-m, which occurs mainly in the bulk of the membrane, was unchanged. Adapted from [19]. Data derived from [39–42].

Heparin coating of dialysis membrane

Control of mural clotting can be accomplished by the use of anticoagulants. Some naturally occurring anticoagulants, such as antithrombin III, contribute to the mixture of proteins adsorbed onto the membrane surface. They are, however, quantitatively insufficient for preventing clotting. Heparin, a complex carbohydrate, is commonly used in haemodialysis [43]. The presence of sulfate groups in the carbohydrate moiety gives heparin its highly negative charge. Heparin binds to antithrombin III, accelerating the enzyme-neutralizing effect of this serine protease inhibitor, and prevents thrombin formation. In the presence of heparin, the interaction of antithrombin with thrombin is virtually instantaneous. The heparin–antithrombin complex inhibits the conversion of other coagulation proteins to active serine proteases (Factors XII, XI, IX and X).

The binding of unfractionated heparin binding to polymers has been extensively studied in an effort to render dialysis membranes more haemocompatible. The binding of heparin shares chemical characteristics with the binding of other blood components, through ionic and covalent interactions. Surface-coating techniques have been developed, but information about these procedures is covered by manufacturers’ patents. For efficiently binding heparin to cellulosic dialyzers, the membrane must first be coated with an intermediate polymer. As a bridging element, polyethylene glycol has been extensively tested [44–49]. This approach has improved the haemocompatibility of artificial devices used for extracorporeal circulation in cardio-pulmonary surgery, but has not been very successful in haemodialysis. More recently, a technique—stretching PEI over the surface of a polycrylonitrile polymer—has produced a new membrane, the AN69-ST membrane. In comparison with the parent AN69 membrane, the new AN69-ST membrane is characterized by a less electronegative surface, while the sulfonic charges present in the body of the membrane have not been modified by the apposition of PEI over its surface [19], as indicated previously. Heparin binding has been documented both in vitro and in vivo [50]. Once adsorbed onto the membrane, heparin keeps its anticoagulant properties. This property presently is used mainly at the bedside, allowing the tapering of heparin doses in haemodialysis, by between half to two-thirds of the regular doses. In some patients at high risk of bleeding and under strict supervision, haemodialysis has been managed without systemic administration of heparin [51]. One may wonder if decreasing heparin doses in the chronically haemodialyzed patient could decrease the risk of heparin-related osteoporosis. Similarly, heparin reduction could decrease hypertriglyceridaemia and prevent the increase of plasma very low density lipoprotein and immediate density lipoprotein particles as well as of hyperlipoprotein(a) found in some 80% of chronically haemodialyzed patients, as these anomalies are associated with the heparin-induced decrease of lipoprotein lipase activity [52,53]. These hypotheses remain to be proven. Binding of heparin should prevent further denaturation and hence activation of the adhered proteins and blood cells [54]. This hypothesis, if valid, should perfectly fulfil the requirements of biocompatibility.

Membrane biocompatibility, nutrition and atherosclerosis

The metabolic consequences of haemodialysis performed with a non-biocompatible membrane have been evaluated. In a pioneer study, Berström et al [55] showed that sham haemodialysis in healthy students induced a significant increase of protein breakdown when cuprophan membrane was used but this harmful effect was blunted when using the polycrylonitrile AN69 membrane. (In both groups, subjects were exposed to a similar dialysate.) The effect could not be ascribed either to amino acid loss during dialysis, as recently confirmed [56], or to backfiltration of a contaminated dialysate.

It is not presently possible to delineate the biological mediators that support the accelerated vascular disease manifested by the haemodialyzed patient. The contributions of dialysis membrane or technique, or of dialysate quality, to the inflammatory status accompanying haemodialysis have been delineated in a recent comprehensive review by Kaysen [57]. Some 50–60%, if not more, of mortality in chronically haemodialyzed patients results from cardiovascular disease. Among many effects triggered by haemodialysis, the activation of nucleated cells releasing cytokines and growth factors on one hand and generating oxidative stress-associated peroxidation and glycation of proteins and lipids on the other, may contribute to accelerated atherosclerosis [58–60]. Increased acute phase proteins, especially C-reactive protein, presently are the best biological markers of inflammation. They reflect the diffuse activation of endothelium, which may also be measured by specific markers derived from endothelial cells, such as E-selectin and sICAM-1. Ridker et al [61] and Koeng et al [62] provided convincing
evidence that the baseline plasma concentration of C-reactive protein in apparently healthy subjects is predictive of cardiovascular events. Therefore, for these findings to be applied to the haemodialyzed patient, we must take into account membrane biocompatibility, whatever the mechanisms, which make a membrane biocompatible and haemocompatible. Supporting this recommended approach are clinical data that suggest an antiinflammatory effect of low doses heparin used during haemodialysis [63].

Conclusion: adsorption as a specific characteristic of biocompatible dialysis membranes

Criteria of biocompatibility should be taken into consideration when using high purity dialysate. As discussed, several biological pathways, stimulated during haemodialysis, are relevant to any definition of membrane biocompatibility. Enzymes and substrates that characterize these pathways interact together, before and after dialysis membranes are coated by plasma proteins. The relationship between the initial phases of classic complement activation and the contact phase process and fibrinolysis have been described. Plasmin activation, which governs fibrinolysis also stimulates the complement cascade either directly or through cleavage of Factor XII. Decreasing membrane binding of complement, Factor XII, platelets and leukocytes with appropriate redistribution of electric charges over the surface and within the body of the membrane appears to be the most efficient way for reducing the inflammatory stress of haemodialysis and rendering dialysis membranes more biocompatible. It is possible that in the near future heparin-coated devices that are suitable for chronic haemodialysis and lessen the activation of cellular and humoral mechanisms of haemoincompatibility, will prove to be reliable for regular use. Biocompatibility of haemodialysis membranes nowadays presents the most important challenge to overcome for improving dialysis quality beyond the mechanistic approach of urea removal.

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References
