

Effect of membrane composition and structure on solute removal and biocompatibility in hemodialysis

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Effect of membrane composition and structure on solute removal and biocompatibility in hemodialysis. Significant changes in extracorporeal membranes have occurred over the past five decades in which hemodialysis (HD) has been available as a therapy for both acute renal failure (ARF) and end-stage renal disease (ESRD). For cellulosic membranes, these changes have included a reduction in thickness, hydroxyl group substitution, and an increase in pore size. These modifications have resulted in enhanced efficiency of small solute removal, a broader spectrum of overall solute removal, and an attenuation of complement activation in comparison to the thick, unsubstituted cellulosic membranes of low permeability used in the early days of HD therapy. Synthetic membranes, originally developed specifically for use in high-flux HD and hemofiltration, have also evolved during this same time period. In fact, the initially clear distinction between low-flux regenerated cellulosic and high-flux synthetic membranes has become blurred, as membrane formulators have developed products designed to appeal to enthusiasts for both membrane formats. The purpose of this review is to characterize both the solute removal and biocompatibility characteristics of dialysis membranes according to their composition (that is, polymeric makeup) and structure. In this regard, the manner in which membrane biocompatibility interacts with flux is highlighted.

Over the past five decades, hemodialyzers used for acute and chronic renal replacement therapy have continuously evolved. Utilization of regenerated cellulose (that is, unsubstituted cellulosic) membranes of small apparent pore size and large thickness in dialyzers with inefficient mass transfers characteristics characterized the very early days of hemodialysis (HD) [1–3]. In fact, coil designs with regenerated cellulose tubes were used almost exclusively throughout the 1960s. Therefore, inefficient small solute removal, negligible middle molecule removal,

and a high degree of complement activation were the norm. The efficiency of small solute removal by unsubstituted cellulosic dialyzers improved markedly in the late 1960s with the introduction of modified flat-plate dialyzers [4], which significantly decreased blood compartment mass transfer resistance. Solute mass transfer was further improved in the early 1970s when cellulosic hollow fiber membranes [5] began to be offered. Also occurring in this decade was the development of synthetic membranes, such as polyacrylonitrile (PAN) and polysulfone [6]. These latter membranes differed fundamentally from regenerated cellulose not only in their polymeric composition but also in a number of other features, including pore size, thickness, and hydrophobicity.

Since these early days of dialysis therapy, additional membranes have been introduced into both the cellulosic and synthetic classes. An initially clear distinction between low-flux regenerated cellulosic and high-flux synthetic membranes has, however, become blurred as membrane formulators have developed products designed to appeal to enthusiasts for both membrane formats. The purpose of this article is to provide an overview of current dialysis membrane technology, emphasizing the manner in which membrane composition and structure influence both solute removal capabilities and biocompatibility properties.

PERMEABILITY CLASSIFICATION OF DIALYSIS MEMBRANES

Although numerous classification schemes have been proposed [7], HD membranes are traditionally classified according to water flux, a term synonymous with water permeability. The clinical parameter used to characterize the water permeability of a dialyzer is the ultrafiltration coefficient (K_{UF} : ml/hr/mm Hg). The water permeability of a dialyzer is usually derived from *in vitro* experiments in which bovine blood is ultrafiltered at varying transmembrane pressures (TMPs). The relationship between plasma ultrafiltration rate and TMP is linear at relatively

Key words: acute renal failure, end-stage renal disease, cellulosic dialysis membranes, synthetic dialysis membranes, hemofiltration, biocompatibility, water flux.

Received for publication November 6, 1998
and in revised form February 12, 1999
Accepted for publication April 7, 1999

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low TMP values for all membranes, whereas a plateau in ultrafiltration rate occurs at relatively high TMP values [8]. Dialyzer K_{UF} is defined by the slope of the linear portion of this ultrafiltration rate versus TMP curve. The membrane characteristic having the largest impact on water permeability is pore size. As discussed by Lysaght [6], ultrafiltrate flux (ultrafiltration rate per unit area of membrane) is roughly proportional to the fourth power of the mean membrane pore radius. As such, small changes in pore size have a very large effect on water permeability.

A common misconception relating to dialyzer performance is the assumption that a membrane's solute removal capabilities are necessarily correlated with its water permeability. Diffusion, the predominant mass transfer mechanism in HD, can be characterized by solute flux (ϕ : mass removal rate per unit surface area of membrane). Based on a model in which a membrane has N (straight) cylindrical pores (per unit membrane surface area) of radius r , diffusive solute flux can be expressed as [6] follows:

$$\phi = \lambda D \rho \Delta C / t \quad (\text{Eq. 1})$$

where λ is the solute partition coefficient; D is solute diffusivity; ρ is membrane porosity; ΔC is the transmembrane concentration gradient, and t is membrane thickness. (While the partition coefficient is essentially unity for solutes such as urea and creatinine, larger solutes with incomplete access to the membrane pores have λ values that are less than one.) Membrane porosity is a function of both pore size and number:

$$\rho = N \pi r^2 \quad (\text{Eq. 2})$$

Equations 1 and 2 suggest that diffusive transport is relatively favorable for low molecular weight solutes not only because of the inverse relationship between molecular weight and diffusivity, but also because of the greater access of small solutes to the membrane pore structure. Equation 1 also indicates that diffusive transport is enhanced at low values of membrane thickness.

The dissociation between solute and water permeability for dialysis membranes can be explained by use of the previously mentioned fundamental principles. For all dialysis membranes, small solutes such as urea and creatinine have free pore access ($\lambda = 1$). Therefore, small solute transport is highly dependent on membrane porosity. As equation 2 indicates, one membrane with a large number of relatively small pores and a second membrane with a small number of relatively large pores can have equivalent porosities. Although the small solute transport properties of these two hypothetical membranes would be equivalent, the flux (water permeability) properties would greatly differ. This difference is explained by the strong dependence of ultrafiltrate flux on membrane pore size (described earlier in this article).

The potential dissociation between water and solute flux is demonstrated specifically by a Gambro copolymer membrane containing both hydrophobic (polycarbonate) and hydrophilic (polyether) domains [9]. Although strictly defined as a synthetic, this membrane's unique composition also endows it with some properties that are more characteristic of the cellulosic class. The *in vitro* vitamin B₁₂ clearance for a 1.1 m² dialyzer of this type (K_{UF} 6.1 ml/hr/mm Hg) is 87 ml/min [10], and the clinical use of this dialyzer achieves a significant (approximately 20 to 30%) reduction in serum β_2 -microglobulin (β_2 m) concentration [11, 12]. In contrast, unsubstituted cellulosic, cellulose acetate, and polysulfone dialyzers with roughly the same K_{UF} and surface area have *in vitro* vitamin B₁₂ clearances of approximately 50 ml/min and are unable to achieve a significant reduction in serum β_2 m concentration in the clinical setting [11]. Ward et al have recently suggested that the mechanism by which this membrane can remove β_2 m despite a low water permeability is either by adsorption to the hydrophobic microdomains or by diffusion through the relatively few large pores in the hydrophilic domains [12]. Unmodified cellulosic and cellulose acetate membranes having this same dissociation between water and solute permeability have also been developed recently (discussed later in this article).

The only dialyzer classification scheme recognized by the United States Food and Drug Administration is based on water permeability, with low and high permeability dialyzers having K_{UF} values of <8 and ≥ 8 ml/hr/mm Hg, respectively. This scheme is at odds with that used primarily in clinical practice in which dialyzers are divided into low-flux, high-efficiency, and high-flux categories. This categorization is based primarily on solute permeability properties. The National Institutes of Health Hemodialysis (HEMO) Study [13] defines low-flux and high-flux dialyzers as those having a β_2 m clearance of less than 10 ml/min and greater than 20 ml/min during first use, respectively. (An additional high-flux requirement is a $K_{UF} > 14$ ml/hr/mm Hg.) The term "high efficiency" actually had its origin as a therapy, first described by Keshaviah et al [14], rather than a specific type of dialyzer. These investigators employed large surface area cellulose acetate dialyzers and relatively high blood flow rates to achieve sufficiently high urea clearances to allow reductions in treatment time. The use of large surface area dialyzers capable of achieving these high urea clearances has increased to the point that a separate, albeit somewhat indistinct, class of high-efficiency dialyzers now exists. Although this class is defined primarily by its solute removal capabilities, dialyzers in this category generally have K_{UF} values falling in the 8 to 15 ml/hr/mm Hg range, essentially bridging the gap between low-flux and high-flux dialyzers.

BASIC CHARACTERISTICS OF CELLULOSIC MEMBRANES

The monomeric subunit of cellulosic membranes is cellobiose, a naturally occurring saccharide found in plants [4]. Chemically, cellobiose is a ringed structure richly endowed with hydroxyl groups. The interaction of complement cascade products with these hydroxyl groups is felt to be responsible, at least partly, for the relatively pronounced complement activation and leukopenia observed when unsubstituted cellulosic membranes contact blood [15]. For the past several years, a major objective among manufacturers has been the development of modified (substituted) cellulosic membranes in which a certain fraction of these hydroxyl groups is replaced with other moieties. The substitution groups diminish the degree of complement activation by at least three different mechanisms. One mechanism is the replacement of a large percentage of the hydroxyl groups with an acetate radical [5]. (Although the hollow fiber membrane produced by Dow in the 1970s was extruded as cellulose acetate, it functionally was regenerated cellulose due to a de-esterification step in the manufacturing process.) In the first truly substituted cellulosic membrane, cellulose (di)acetate, approximately 70 to 80% of the hydroxyl groups on the cellulosic backbone were replaced with an acetate group. Most likely because this modification eliminates a large fraction of the active surface sites for interaction with complement components, an attenuation of the intense complement activation seen with unmodified cellulose was achieved. This membrane modification also resulted in a moderate increase in pore size, yielding a slightly higher water permeability and broader solute removal spectrum for cellulose acetate in comparison to unsubstituted cellulosic membranes of similar surface area [14]. Extrapolation of this process to total replacement of the hydroxyl groups resulted in the cellulose triacetate fiber characterized by further attenuation of complement activation and higher water permeability [16]. Recent data demonstrate the biocompatibility profiles for high-flux cellulose triacetate and polysulfone dialyzers are comparable [16–18].

A second cellulosic substitution mechanism is the replacement of a relatively small percentage (less than 5%) of the hydroxyl groups with a bulky chemical group, which sterically reduces the degree of interaction between complement activation products and the membrane. Examples for which this strategy is employed are Hemophan® (tertiary amine substitution) [19, 20] and synthetically modified cellulose (SMC; benzyl substitution group) [21]. A final mechanism by which complement activation can be attenuated is modulation of factors D and H, which act as an up-regulator and a down-regulator, respectively, in the complement cascade [22, 23]. Although this mechanism is most relevant for synthetic

membranes because of their ability to adsorb these factors nonspecifically, it appears to play a role for Hemophan® also [24]. Finally, a vitamin E-modified cellulosic membrane has also been introduced recently [25].

The SMC membrane, the polycarbonate/polyether membrane, and certain synthetic membranes, are examples of surfaces endowed with microdomain structures. As discussed recently [21, 26], a membrane having a combination of hydrophilic and hydrophobic domains rather than a uniformly hydrophilic or hydrophobic composition has theoretical advantages with respect to biocompatibility. Although hydrophilic surfaces promote complement activation, hydrophobic surfaces tend to be thrombogenic because of their ability to adsorb proteins and activate platelets [27]. The purposeful interspersing of hydrophilic and hydrophobic domains in a “checkerboard” pattern is designed to strike a balance between complement activation and thrombogenicity and to enhance the overall biocompatibility of the previously mentioned membranes.

The evolution in cellulosic membranes has resulted in a wide spectrum of biocompatibility and flux profiles. If complement activation and neutropenia are used as the major biocompatibility criteria [28, 29], regenerated cellulose is the least biocompatible, whereas cellulose triacetate is the most biocompatible, with the other modified cellulosic membranes having intermediate profiles. However, characterization of the flux properties of these membranes is not as straightforward. For dialyzers of comparable surface area, a simplistic approach is to report K_{UF} values in the following ascending order: regenerated cellulose < Hemophan®, SMC < cellulose acetate < cellulose triacetate [10, 30]. In this simplistic scheme, a 1.5 m² dialyzer having a regenerated cellulose, Hemophan® or SMC membrane generally falls in the low-flux category (K_{UF} < 8 ml/hr/mm Hg), whereas comparably sized dialyzers having cellulose acetate and cellulose triacetate membranes fall in the mid-flux (K_{UF} 10 to 20 ml/hr/mm Hg) and high-flux (K_{UF} > 20 ml/hr/mm Hg) categories, respectively. However, this simplistic categorization scheme breaks down in several respects. High-flux cellulose acetate membranes have now been produced by two different HD membrane manufacturers [18, 31, 32], and cellulose triacetate dialyzers of low water permeability (K_{UF} 9.5 ml/hr/mm Hg) are also available [33]. Finally, the recent development of unmodified cellulosic and cellulose acetate membranes having relatively low water permeability but solute removal capabilities that include β_2m [34, 35] further confounds this classification scheme and provides additional examples of a dissociation between water and solute flux. Thus, as is the case with synthetic membranes, neither the water nor solute permeability properties of a cellulosic HD membrane are defined by its specific polymeric composition.

BASIC CHARACTERISTICS OF SYNTHETIC MEMBRANES

The monomeric subunits of the various synthetic membranes individually vary, and all differ significantly from cellobiose. The absence of surface hydroxyl groups on synthetic membranes is one factor responsible for the reported differences in complement activation between synthetic membranes and either unsubstituted cellulosic membranes or modified cellulosic membranes of low permeability. Subsequent to the introduction of the AN69® membrane in the early 1970s, numerous synthetic membranes have been introduced for clinical use. Similar to AN69®, polysulfone and polyamide were brought to the market for use in both high-flux HD and hemofiltration [36, 37]. One obvious reason accounting for the use of these membranes in a hemofiltration mode is their significantly larger pore size and higher hydraulic permeability than regenerated cellulose membranes. The other reason relates to the structural differences between the synthetic and unsubstituted cellulosic membrane groups. Cellulosic membranes have relatively thin walls (generally in the 6 to 15 μm range), which have a uniform (symmetric) composition across their entire thickness. Although the relative thinness of cellulosic membranes is desirable with respect to diffusive solute transport (discussed later in this article), this same characteristic renders many cellulosic membranes unable to withstand the high ultrafiltration rates required to perform convective therapies. The synthetic membranes have thicker walls (20 μm or more) that may be structurally symmetric [for example, AN69®, polymethylmethacrylate (PMMA)] or asymmetric (for example, polysulfone, polyamide). In the latter category, a very thin "skin" (approximately 1 μm) contacting the blood compartment lumen acts primarily as the membrane's separative element with regard to solute removal, whereas the remaining thickness (stroma) imparts mechanical strength. In turn, the composition of the stroma layer is quite variable for the various synthetic membranes [37]. For the Fresenius polysulfone membrane (Fresenius Medical Care, Bad Homburg, Germany), the stroma is relatively homogeneous with a sponge-like structure, whereas the Gambro polyamide membrane (Gambro Renal Care, Hechingen, Germany) has, adjacent to the skin, a sponge-like stroma layer that has progressively larger pores ("macrovoids" with a finger structure) in the radially outward direction. Finally, a new synthetic (polyethersulfone) membrane developed by Membrana GmbH (formerly Akzo Nobel) (Wuppertal, Germany) has a novel configuration consisting of a sponge-like stroma layer interposed between skin layers on both the inner (blood-side) and outer (dialysate-side) aspects (abstract; Kayser et al, *J Am Soc Nephrol* 8:163A, 1997).

In the production of synthetic membranes made of primarily hydrophobic polymers (polysulfone, polyamide,

polyethersulfone), a hydrophilic additive [polyvinylpyrrolidone (PVP)] acts as a polymer alloy. PVP is used to impart sufficient hydrophilicity to the membrane to allow clinical use and, as a wetting agent, modulates surface tension and viscosity within the pore structure during membrane formulation. This latter feature explains PVP's importance in determining the overall pore size distribution of synthetic membranes.

Although synthetic membranes are employed for both hemofiltration and high-flux HD, it is in the latter mode that these membranes have found their widest application. Another synthetic membrane formulation was reported in the late 1980s with the introduction of low-flux versions. Low-flux polysulfone [38] and PMMA [12] have been used clinically for several years now, and recently, a low-flux version of a polyamide/polyethersulfone copolymer has been introduced (abstract; Kaiser et al, *Blood Purif* 16:236, 1998). Overall, the percentage of the American HD market employing polysulfone membranes has increased from approximately 10% in the late 1980s to approximately 50% currently. However, in recent years in the United States, the rate of growth in the use of polysulfone has been more rapid for the low-flux version than the high-flux version.

EFFECT OF MEMBRANE COMPOSITION AND STRUCTURE ON DIALYTIC SOLUTE REMOVAL

One classification scheme for uremic solutes is as follows: small solutes (molecular wt < 200 Da), most of which are nitrogenous in nature, middle molecules (molecular wt 500 to 2000 Da), and low molecular wt oligopeptides and proteins (molecular wt 2000 to 50,000 Da).

Small solute removal

Small solute removal during HD occurs almost exclusively by diffusion. To quantify a particular membrane's diffusive capabilities, its mass transfer resistance is frequently used [4]:

$$R_O = R_B + R_M + R_D \quad (\text{Eq. 3})$$

In equation 3, the overall resistance to diffusive mass transfer of a particular solute (R_O) has three components: blood compartment resistance (R_B), resistance caused by the membrane itself (R_M), and dialysate compartment resistance (R_D). Minimizing the mass transfer resistance in the blood compartment primarily requires the use of relatively high flow rates (that is, shear rates) that decrease unstirred layers. Dialysate-side mass transfer resistance is likewise decreased by increasing the flow rate, but optimal dialysate perfusion of fiber bundles is also a consideration. Although increasing the dialysate flow rate may itself improve fiber bundle perfusion (discussed later in this article), another mechanism by which this

can be achieved is the inclusion of spacer yarns. These devices are spacing filaments placed externally to the fibers and are designed to facilitate dialysate distribution and to reduce channeling [39]. The resistance related to the membrane itself actually has two components:

$$R_M = X_M/D_M \quad (\text{Eq. 4})$$

where X_M is the effective diffusion path length for a solute, and D_M is the solute-specific membrane diffusivity. This equation indicates that a decrease in membrane resistance can be achieved either by a decrease in membrane thickness or an increase in membrane diffusivity.

A recent study published by Murthy et al clearly illustrates the concept that a membrane's small solute removal capabilities are not necessarily tied to its water permeability [35]. Six chronic HD patients were treated with a large surface area unsubstituted cellulose dialyzer (2.2 m²; K_{UF} , 6 ml/hr/mm Hg) and a high-flux polysulfone dialyzer (1.8 m²; K_{UF} , 52 ml/hr/mm Hg) in a crossover manner. The mean first-use urea clearance was higher, although not significantly, for the cellulose dialyzer at a blood flow rate of both 300 ml/min (246 ± 6 vs. 241 ± 2 ml/min) and 400 ml/min (288 ± 8 vs. 280 ± 4 ml/min). This equivalence was achieved despite the substantially greater pore size and higher water flux of the polysulfone membrane. Although the greater surface area of the cellulose dialyzer contributed to this equivalence, the same type of cellulose dialyzer with a comparable surface area (1.7 m²) has an *in vitro* urea mass transfer coefficient (permeability-area product: KoA) that is substantially greater than that of the 1.8 m² polysulfone dialyzer studied (1030 and 945 ml/min, respectively) [10]. These findings suggest the significantly lower thickness (9 μm) of the cellulose dialyzer relative to that of the polysulfone dialyzer (40 μm) was an important factor in this study.

Additional recent data emphasizing the importance of membrane thickness in small solute removal were published by Leyboldt et al [40], who determined *in vitro* urea KoA values for highly permeable cellulose triacetate (1.9 m²; K_{UF} , 36 ml/hr/mm Hg) and polysulfone (1.8 m²; K_{UF} , 55 ml/hr/mm Hg). For blood flow rates ranging between 300 and 450 ml/min and a dialysate flow rate of 500 ml/min, the mean urea KoA value for the cellulose triacetate dialyzer (1070 ml/min) was approximately 43% higher than that of the polysulfone dialyzer (750 ml/min). An increase in the dialysate flow rate to 800 ml/min resulted in a 15% increase in urea KoA for both dialyzers, preserving the difference between the dialyzers on a percentage basis. For these dialyzers of comparable water flux, the probable explanation for this difference was, again, the large difference in membrane thickness (15 vs. 40 μm, cellulose triacetate vs. polysulfone).

Middle molecule removal

Vitamin B₁₂ (molecular weight, 1350 Da) is commonly used for *in vitro* characterizations of dialyzers [41]. How-

ever, because of its extensive binding to plasma proteins, this compound is not useful *in vivo*. In fact, the removal of uremic solutes having molecular weights that fall in the classic middle molecule category has been difficult to quantify because of the lack of an easily measured *in vivo* surrogate molecule. Because recent evidence suggests that uremic appetite suppression is mediated by the retention of a solute(s) in this size range [42], an understanding of removal mechanisms for middle molecules is important. Based on dialysis practices used in the 1960s and early 1970s (that is, relatively low flow rates and thick, low permeability cellulosic membranes), diffusive middle molecule removal was so limited that any convective removal contributed relatively substantially to total removal [41]. However, the situation is vastly different in contemporary HD, in which higher flow rates and dialyzer membranes of significantly greater diffusive permeability for middle molecules are employed.

Agarwal and Cronin assessed middle molecule removal by two high-efficiency dialyzers for seven patients studied in a crossover manner [43]. They studied gentamicin, a reasonable middle molecule surrogate in light of its appropriate molecular weight (518 Da), minimal protein binding, and small volume of distribution. A variety of gentamicin removal parameters was determined for an unsubstituted cellulose dialyzer (1.75 m²; K_{UF} , 6.0 ml/hr/mm Hg; thickness, 9 μm) and polysulfone (1.8 m²; K_{UF} , 8.1 ml/hr/mm Hg; thickness, 40 μm). For both dialyzers, an essentially linear relationship between clearance and blood flow rate was observed, suggesting diffusion was the predominant removal mechanism. At blood and dialysate flow rates of approximately 275 and 500 ml/min, respectively, gentamicin clearance (58.2 ± 8.0 vs. 41.7 ± 6.9 ml/min) and gentamicin KoA (127.3 ± 6.3 vs. 84.6 ± 4.7 ml/min) were significantly greater for the cellulose dialyzer. Finally, for the cellulose dialyzer, these investigations produced a significant linear relationship between urea Kt/V and gentamicin Kt/V ($r = 0.87$, $P = 0.01$). Collectively, these data demonstrate middle molecule removal by the more efficient dialyzers used in contemporary HD is primarily achieved by diffusion. As such, the use of treatment parameters that favor diffusive solute removal, such as high flow rates and thin membranes, is expected to produce a concomitant increase in middle molecule removal.

Thalhammer et al recently measured dialytic removal parameters for another drug, ofloxacin, whose molecular weight (361 Da) also falls in the middle molecule range [44]. In a total of 13 patients studied in a noncrossover manner, ofloxacin clearance and percentage reduction were measured for polysulfone (1.3 m²; K_{UF} , 8.5 ml/hr/mm Hg) and cellulose acetate dialyzers (1.5 m²; K_{UF} , 6.0 ml/hr/mm Hg). (The measurements were made during a period in which the drug was administered 100 mg daily by mouth.) Mean treatment characteristics included a

blood flow rate of approximately 250 ml/min and dialysate flow rate of 500 ml/min. Arteriovenous clearance (108.4 ± 15.8 vs. 92.4 ± 39.4 ml/min; polysulfone vs. cellulose acetate) and percentage reduction normalized to membrane surface area (49.6 ± 5.8 vs. $45.5 \pm 4.8\%/m^2$) did not differ significantly. However, arteriovenous clearance normalized to the membrane surface area was significantly higher for the polysulfone dialyzer (5.0 ± 0.7 vs. 3.7 ± 1.6 l/hr/ m^2 , $P < 0.05$).

We have recently reported findings for middle molecule removal by high-flux membranes [45]. The molecular weight (1448 Da), protein binding, and volume of distribution of vancomycin, another antibiotic commonly used in the acute renal failure (ARF) and end-stage renal disease (ESRD) populations, enable this drug also to be a uremic middle molecule surrogate [46, 47]. We measured vancomycin removal by a cellulose triacetate dialyzer (1.9 m^2 ; K_{UF} , 36 ml/hr/mm Hg) in eight patients who received 1 g of vancomycin intravenously during the last hour of dialysis. The mean blood and dialysate flow rates were 423 and 600 ml/min, respectively. A significant linear correlation ($r = 0.88$, $P < 0.005$) was observed between the percentage removal of vancomycin and a measure of diffusive small solute removal, urea Kt/V . In addition, diffusion was determined to account minimally for a mean of 92% of total vancomycin removal. In a subsequent *in vitro* study [48], we assessed the mass transfer of vancomycin and another widely studied large molecule surrogate, inulin (molecular weight, 5200 Da), for high-flux cellulose triacetate (1.9 m^2 ; K_{UF} , 36 ml/hr/mm Hg) and polysulfone (1.8 m^2 ; K_{UF} , 52 ml/hr/mm Hg) dialyzers. This study corroborated the above clinical data for vancomycin and also suggested that diffusion is the predominant mass transfer mechanism, even for solutes as large as inulin, for both high-flux dialyzers. The mean first-use inulin clearance was found to be significantly higher for cellulose triacetate versus polysulfone (76 vs. 43 ml/min, respectively, $P < 0.05$). The lower diffusive mass transfer resistance of the thinner cellulose triacetate membrane may account for these findings. However, another possible explanation may relate to the pore size dimensions of the polysulfone dialyzer specifically used in the study. This dialyzer, which is designed for units employing bleach-based reprocessing, has a relatively low first-use β_2m clearance, which increases significantly when it is reprocessed with bleach [13]. In our study [48], inulin clearance for the polysulfone dialyzer also increased significantly during bleach reprocessing, such that no significant difference was observed between the two dialyzers after 10 *in vitro* reuses. Thus, a difference in mean pore size may also explain the first-use difference for inulin clearance in our study. Of note, relative to the polysulfone dialyzers used in this study, recent data [15] indicate the first-use β_2m clearance of more recently manufactured dialyzers is higher.

These recent data demonstrate that, based on the operating conditions and dialyzers used in contemporary HD, middle molecule removal occurs predominantly by diffusion. However, middle molecule removal can be significantly enhanced by convective therapies, such as hemofiltration and hemodiafiltration, in which an absolute ultrafiltration rate in excess of that required for plasma volume reduction is employed [49–51]. In general, the degree to which convection augments total solute removal is proportional to solute molecular weight due to the inverse relationship between solute diffusivity and size [52].

Low molecular weight protein removal

Recent interest in increasing the extracorporeal removal of β_2m has provided insight into the general mechanisms mediating the removal of low molecular weight proteins. A number of studies published in the past 15 years [11, 52–63] support several general conclusions. First, β_2m removal by low-flux unsubstituted cellulosic membranes is usually negligible, although certain exceptions do exist [35]. Second, the primary mechanism by which β_2m is removed during high-flux HD varies widely among membranes. For certain membranes, such as sulfonated PAN (AN69®) and particularly PMMA, removal is achieved predominantly or solely by adsorption. At the other end of the spectrum is the cellulose triacetate membrane, for which adsorption is minimal and removal occurs primarily by diffusion. High-flux polysulfone and unsulfonated PAN membranes have intermediate adsorptive characteristics and achieve transmembrane β_2m removal by a combination of diffusion and convection. Third, at least for the high-flux synthetic membranes, enhanced convective removal by the use of high ultrafiltration rates (with hemofiltration or hemodiafiltration) increases β_2m removal relative to standard (diffusion-based) HD. Although many clinicians consider β_2m to be a surrogate for the low molecular weight protein class of uremic solutes, this assumption has not been conclusively proved. Nevertheless, it is reasonable to use the abundant transport data available for β_2m to provide insight into the transport characteristics of other low molecular weight proteins, such as complement activation products and cytokines.

We recently measured the protein transport properties of high-flux membranes. In an initial set of studies [64], fragments of AN69® membranes were incubated in saline containing ^{125}I -labeled β_2m . Adsorption isotherms (amount of protein adsorbed vs. concentration in solution at equilibrium) [65] were generated for both porous (commercially available) AN69® and a nonporous preparation of the same polymer. At equivalent solution concentrations, the β_2m binding affinity of the porous PAN was approximately eight times greater than that of the nonporous PAN. These data indicate that the adsorptive

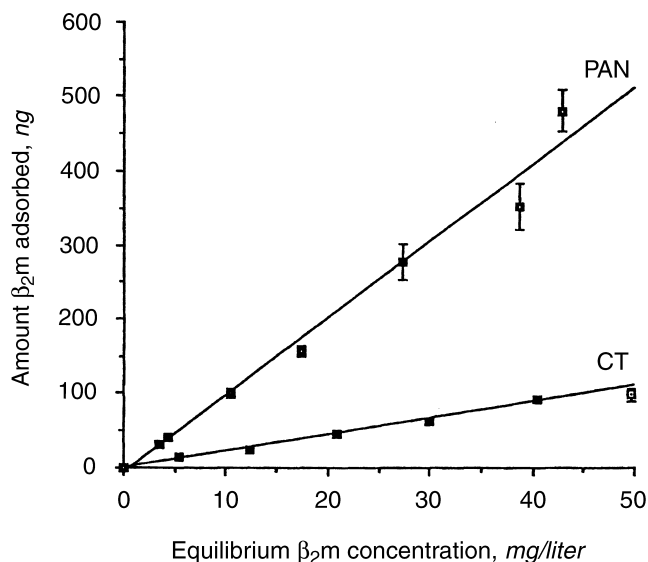


Fig. 1. Isotherm curves for β_2 -microglobulin (β_2m) adsorption to polyacrylonitrile (PAN) and cellulose triacetate (CT). The relationship is linear for both membranes. The slope of the PAN line is approximately fivefold greater than that of the CT line. (Reprinted with permission from Clark et al and the International Society of Nephrology [66].)

surface area resides primarily in the pore structure of a high-flux HD membrane rather than the nominal surface area. As such, the adsorption of a low molecular weight protein is highly dependent on access of the protein to a membrane's internal pore structure. Consequently, adsorption of low-molecular weight proteins, such as C3a and β_2m , to low-flux membranes is not expected to be clinically significant, at least in comparison to that which occurs to high-flux membranes.

We also compared protein removal mechanisms for different high-flux membranes [66]. Based on the slopes of the equilibrium isotherm curves, we found that the adsorption affinity of AN69[®] for β_2m was approximately five times that of the cellulose triacetate membrane (Fig. 1). This finding was attributed to the greater hydrophobicity of the synthetic membrane. However, the β_2m diffusive mass transfer resistance of the AN69 membrane was found to be approximately 1.7-fold greater than that of cellulose triacetate, a result that translated into an order of magnitude difference in the β_2m membrane diffusion coefficients (3.25×10^{-7} vs. 0.30×10^{-7} cm²/sec; cellulose triacetate vs. AN69[®]). Thus, in terms of β_2m (and presumably other low molecular weight protein) removal, our findings corroborate previous experimental and clinical data demonstrating the high adsorptive affinity of the sulfonated PAN membrane [19, 67]. However, our data also suggest that this membrane is not very well suited for diffusive removal of uremic solutes in this class, at least in comparison to high-flux cellulosic membranes.

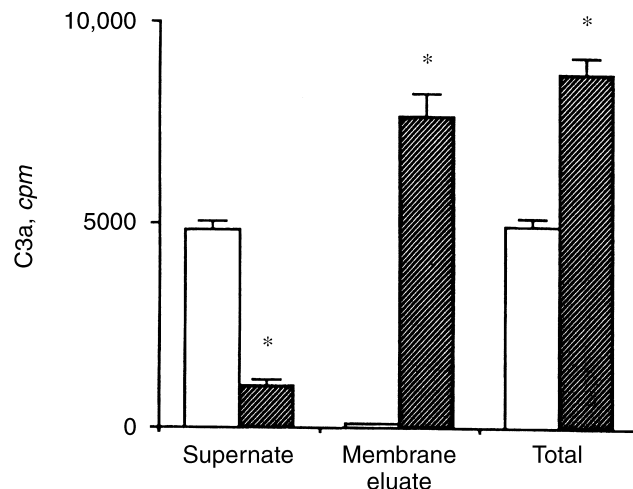


Fig. 2. Generation of C3a during incubation of serum with cuprophane or PAN membranes. Symbols (\square cuprophane; \blacksquare PAN) depict the mean \pm SEM ($N = 5$). *The difference between cuprophane and PAN is statistically significant ($P < 0.001$). (Reprinted with permission from Cheung et al [67] and the International Society of Nephrology.)

INTERACTION BETWEEN BIOCOMPATIBILITY AND FLUX

Measurement of complement pathway byproducts is one technique used to assess the inflammatory response elicited by exposure of blood to a dialysis membrane. However, numerous previous studies have failed to account for the fact that the clinically measured complement components (C3a and C5a) are low molecular weight proteins. Therefore, the concentration of these inflammatory mediators represents the net result of the simultaneous processes of generation and any dialytic removal that may occur. In this regard, complement activation products are similar to most uremic solutes, for which both generation and net removal need to be considered [68]. The corollary of this observation is that the permeability properties and not just the polymeric composition of a dialysis membrane must be considered when evaluating complement activation data.

The effect of simultaneous generation and removal on net complement activation was investigated in a classic study published by Cheung et al [67]. Dialysis membrane fragments composed of either (low-flux) regenerated cellulose (Cuprophane[®]) or (high-flux) PAN (AN69[®]) were incubated in plasma containing C3 molecules previously radiolabeled with ¹²⁵I. After a 30-minute incubation period, the generation of C3a (molecular weight, 9.5 kDa) resulting from contact of the plasma with the membranes was measured. For each membrane, total C3a generation was determined by summing the amount in the supernate (fluid phase) and the amount adsorbed to the membrane. The results (Fig. 2) indicate that the total C3a generation was actually greatest for the "biocompatible" AN69[®]. However, nearly all of the C3a generated was rapidly

Table 1. Inflammatory mediators

| Mediator | Molecular weight kD |
|---|------------------------|
| Lipid A | 2–4 |
| Lipopolysaccharide (LPS) fragments | <8 |
| C3a | 8.9 |
| Granulocyte inhibitory peptide (GIP) II | 9.5 |
| C5a | 11 |
| Interleukin-1 | 17 |
| Tumor necrosis factor (monomeric) | 17 |
| Factor D | 23 |
| Granulocyte inhibitory peptide (GIP) I | 28 |
| Tumor necrosis factor (trimeric) | 55 |
| Lipopolysaccharide (LPS) | >100 |

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adsorbed to this hydrophobic membrane. Total C3a generation was significantly lower for the regenerated cellulose membrane, but virtually all of the protein remained in the fluid phase rather than being bound to this hydrophilic surface. In extrapolating these data to clinical HD, a reasonable assumption is that the fluid phase of these experiments corresponds to the venous (dialyzer effluent) blood line in the extracorporeal circuit. Therefore, only that portion of the generated C3a remaining in the fluid phase would reach the bloodstream of the patient to act as a potential inflammatory mediator. These data are useful in interpreting clinical studies in which complement protein concentrations are reported and underscore the need to understand the permeability and solute removal properties of a membrane when evaluating complement activation data.

It is simplistic to limit the discussion about membrane biocompatibility to complement activation, as a number of agents have been identified as potential inflammatory mediators in chronic HD patients. A list of these putative mediators appears in Table 1 [69, 70]. Some of these compounds, such as lipid A and lipopolysaccharide fragments, potentially have their origin in dialysate, a nonsterile fluid [71–73]. Because of their relatively low molecular weight, these inflammatory mediators may undergo transmembrane passage and induce cytokine production in the bloodstream, either directly via an effect on mononuclear cells or indirectly via an effect on the alternative complement pathway [74–78]. Conversely, the majority of the mediators that are potentially elicited in the blood, such as C3a and interleukin-1, may be simultaneously eliminated during high-flux therapies by an adsorptive or transmembrane mechanism, as demonstrated in the Cheung et al study [67]. Other investigations have confirmed that adsorption is also important in the removal of other inflammatory mediators, such as factor D [19] and cytokines [79].

Recent clinical data illustrate these concepts. Muller et al measured serial C3a concentrations during HD with

both low-flux and high-flux polysulfone dialyzers [80]. Peak plasma C3a concentrations were approximately twofold higher with the low-flux than the high-flux membrane. In addition, the area under the C3a versus time curve, which is a time-integrated measure of complement activation, appeared to be significantly higher for the low-flux dialyzer. A similar difference in the extent of complement activation between low-flux and high-flux polysulfone dialyzers has also been reported by Dumler et al (abstract; Dumler et al, *Blood Purif* 10:91–92, 1992). These findings are most likely explained by the ability of the high-flux polysulfone membrane to remove a greater proportion of the generated complement component, relative to the low-flux membrane. The larger mean pore size of the high-flux membrane allows greater pore access of the generated C3a and subsequent removal by a transmembrane (diffusive or convective) or adsorptive mechanism.

Similar reasoning can be applied to a recent study by Hoenich et al [34]. These investigators measured serum C3a concentrations in a group of patients treated with two cellulose acetate membranes of differing pore size and a low-flux polysulfone membrane in a double crossover design. The two cellulose acetate membranes had reported mean maximum pore radii of 43 and 45 Å. Despite the similarity in membrane composition, the peak C3a concentration observed for the larger pore cellulose acetate membrane was significantly less than that of its smaller pore counterpart and was not significantly different from that of low-flux polysulfone. Differences in mean pore size or possibly in pore size distribution with resultant differences in C3a removal capabilities may explain the differences observed for the two cellulose acetate membranes.

Deppisch et al have shown that the generation of another inflammatory marker, the terminal complement complex (TCC), is higher for low-flux than for high-flux polysulfone [81]. Because of its large molecular size, removal of this mediator is not expected to be significant even by a high-flux HD membrane. Therefore, a mechanism other than enhanced removal of TCC itself, possibly one related to complement factor D, most likely accounts for this finding. Factor D is another example of an inflammatory mediator that is differentially removed by low-flux and high-flux HD membranes. This compound is a 23 kDa serine protease whose excretion under normal circumstances is similar to that of β_2m : glomerular filtration followed by proximal tubule reabsorption/catabolism [17, 82]. Therefore, in patients with ESRD, serum concentrations are markedly elevated. This protein acts as an up-regulator of the alternative complement pathway [83], and activation of the alternative pathway by blood–membrane interaction (with resultant C3a generation) is enhanced in the presence of the high serum concentrations of factor D found in uremic patients. Recent data demonstrate that factor D removal is negligible

by low-flux HD but can be significant by hemofiltration, such that the latter can achieve a significant reduction in serum factor D concentrations over time [84]. Preliminary data (abstract; Dupuy et al, *Nephrol Dial Transplant* 8:986, 1993) also indicate that significant factor D removal can be achieved with high-flux HD. Accordingly, the available data suggest that high-flux therapies favorably influence serum C3a profiles (relative to low-flux HD) by at least two mechanisms. One mechanism is a dampening of the alternative complement pathway by enhanced factor D removal, whereas the other is removal of a significant proportion of generated C3a.

SUMMARY

Dialyzers used in contemporary HD are equipped with a wide variety of membranes, and within both the cellulosic and synthetic classes, water and solute flux properties vary widely. Although a direct correlation between water and solute permeability exists for some membranes, these two characteristics can be dissociated for others. For small- and middle-sized solutes, abundant clinical data point to the importance of membrane thickness in diffusive mass transfer. The removal of low molecular weight proteins may occur largely by adsorption for some high-flux membranes, particularly those of hydrophobic synthetic composition. Because many of the mediators of inflammation in dialysis patients fall in this low molecular weight protein category, the biocompatibility of a particular membrane must be interpreted in conjunction with its permeability properties.

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