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Polymeric adsorbent for removing toxic proteins from blood of patients with kidney failure

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Abstract

A hypercrosslinked styrenic polymer with an enhanced proportion of mesopores in the range 2–20 nm has been developed. The principle of the synthesis consists of the suspension polymerization of divinylbenzene (or copolymerization of styrene with divinylbenzene) in the presence of a porogen that is a Θ-solvent for polystyrene. On the scale of thermodynamic affinity, Θ-solvents occupy a border position between good solvents and precipitating media for the growing polymer chains. In this case, microphase separation takes place during the final stages of the polymerization process. The polymer was shown to adsorb 93–98% of β_2-microglobulin from the blood or plasma of patients with chronic kidney failure. At the same time, large essential proteins, like albumin, are not removed to a significant extent, obviously, due to the size-exclusion effect and the difference in the hydrophobicity of the proteins. By replacing surface exposed pendant vinyl groups of the polymer with hydrophilic functional groups, the material was made hemocompatible, according to the standard battery of biocompatibility tests required by ISO 10993 guidelines. No adverse effects such as fever or hypotension were noted in dogs in direct hemoperfusion experiments with the polymer.

Keywords: Toxic proteins

1. Introduction

The human kidney allows for the efficient removal of excess fluid and waste products of metabolism of both low and relatively large molecular masses. Once kidney failure occurs, this function is often replaced with a hemodialyzer or hemofiltration device [1]. The first devices of this type utilized natural cellulose membranes, which possessed predominantly small pores. These membranes permitted the removal of excess fluid, ions and small molecules, but prohibited the removal of substances above $M_r \approx$ 1200 in size. Larger molecules, such as β_2-microglobulin (M_r 11 800), accumulated in the blood and were thought to contribute to many of the additional health problems and high mortality of patients on dialysis. This idea, coined the 'middle molecule hypothesis' by Bapp et al. [2], led to the development of new synthetic dialysis membranes that possessed larger pores and, in combination with equipment to control transmembrane pressure, permitted more efficient elimination of middle molecules. However, several investigators have shown that nonspecific adsorption of middle molecules to the surface of these synthetic 'high-flux' membranes, rather than diffusion through the membrane, can account for significant amounts of the device's clearance [3,4].

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The use of high-flux membranes gained significant support in 1985 when Geyjo et al. [5] conclusively established the link between the accumulation of β_2-microglobulin (β2M) and a complication of long-term dialysis called dialysis-related amyloidosis (DRA). As kidney failure progresses, β2M concentrations in the extracellular compartments increase, often to levels 30–60 times normal. In DRA, insoluble plaques of β2M known as amyloid cause a progressive inflammatory arthropathy of the joints and spine. Patients with DRA often require surgery to correct carpal tunnel syndrome, a common manifestation of DRA, and lifelong medications to ameliorate joint pain and inflammation.

High-flux dialysis membranes achieve ≈23–37% reductions in plasma β2M levels [6]. Several investigators have shown that the use of high-flux membranes can retard, but not prevent, the onset of DRA in dialysis patients [7]. More recently, Port et al. [8] have reported a 5% improvement in patient survival with enhanced middle molecule clearance, though the identity of the toxins responsible for this effect remains unknown. Similar improvements in morbidity and mortality associated with higher clearances of middle molecules have been reported recently by other investigators [9].

In a previous paper [10], we suggested that a more efficient 'artificial kidney' should incorporate both a semi-permeable membrane and an adsorbent cartridge. Whereas filtration through the membrane should remove excess water together with urea and other small toxins, hemoperfusion through the adsorbent bed should remove larger toxins, such as β2M.

A special polymeric adsorbing material (BM-01, Kaneka, Japan) has been described by another group [11] for the selective removal of β2M from the blood of dialysis patients. The adsorbent consists of porous cellulose beads modified with hexadecyl groups that attract β2M through a hydrophobic interaction. The adsorption capacity of this material is 1 mg of β2M per 1 ml of adsorbent. Using a hemoperfusion cartridge containing 350 ml of these cellulose beads in sequence with a high-flux hemodialyzer, several small clinical trials were performed. During 4–5 h of treatment, about 210 mg of β2M were removed, thus reducing the concentration in the blood by 60–70% of initial levels [12–14]. Clinical improvement was partial, but marked in those patients subjected to thrice weekly treatment with this device for a period of 2 months or more. Subjective improvements were seen in joint mobility, joint pain, and nocturnal awakening. In some cases, a marked reduction in the size of bone cysts of the humerus and femur were noted [15]. The use of this device is currently restricted to Japan and concerns over its high cost have largely prevented more widespread use [16].

Therefore, there would seem to be a need for a new cost effective, selective, and high capacity adsorbent for the removal of β2M. We have developed a biocompatible, hypercrosslinked polystyrene-type adsorbent possessing all of these properties. We have conducted extensive in vitro testing of the adsorptive performance and biocompatibility of our polymer. In addition, several successful in vivo hemoperfusion experiments were performed in healthy canines. No adverse effects were noted and excellent biocompatibility and blood chemistries were seen. The present state of the art in the development and testing of the hemoperfusion polymer is reviewed in this contribution.

2. Development of new adsorbing materials for β_2-microglobulin

Hypercrosslinked networks comprise a special type of three-dimensional polymer which forms in an excess of a thermodynamically good solvent and which is conformationally rigid (see review [17] and references therein). Provided that the above two basic requirements are met, the hypercrosslinked network represents a homogeneous openwork molecular construction of low density and high accessibility to small molecules. Distinguishing features of hypercrosslinked polymers are their ability to swell, i.e. significantly increase in volume, in all types of liquid media, including non-solvents for the corresponding linear polymer, and high adsorption capacity. Based on hypercrosslinked polystyrene, a new generation of efficient adsorbing materials emerged [18,19], which are used in large scale industrial adsorption technologies, as well as in analytical chemistry for trace preconcentration [20] and as HPLC column packing materials [21].

Hypercrosslinked polystyrene are prepared

through extensive crosslinking of polystyrene chains by large amounts of bifunctional conformationally-rigid reagents, like *p*-xylilene dichloride, and in the presence of a good solvent, such as ethylene dichloride. Hypercrosslinked polystyrene represents a microporous transparent material. It displays extremely high apparent surface area, up to 1000–1500 m^2/g, and unprecedented adsorption capacity. However, with the average pore diameter of 1–2 nm, the material cannot be used for the adsorption of protein molecules. Some commercially available hypercrosslinked polystyrene materials, like Macronet Hypersol MN-200 [19], possess large transport pores of about 80–100 nm in diameter, in addition to the micropores of 1–2 nm. Adsorbents of this type showed high affinity to β2M and could be made hemocompatible in several different modes [10]. However, the porous structure of these materials was not considered optimal for the adsorption of β2M. The molecular diameter of β2M can be estimated as 3.35 nm, assuming the molecule to be spherical in shape. Thus, polymers with an enhanced proportion of mesopores, in the range 4–10 nm, are needed.

It has been well documented that microphase separation during the formation of a rigid polymeric network results in macroporous materials. Macroporous copolymers of styrene with divinylbenzene (DVB) are the best known materials of this kind. They are prepared by styrene–DVB copolymerization in the presence of an organic diluent that is miscible with the monomers, but causes precipitation of the polymer formed (microphase separation).

On the contrary, microporous structures are created if the formation of the network proceeds in the presence of a diluent which is compatible with both the comonomers and the polymer [17]. No phase separation takes place during such a process. However, since micropores generate extremely high inner surface areas, the final polymers are rich in surface energy and tend to collapse to a nonporous dense material. For this reason, extremely high crosslinking densities and high rigidity of the structure are needed, in order to arrive at a stable microporous hypercrosslinked material.

When taking into account the above regularities, it was logical to suppose that mesoporous networks could form when the diluent represents an organic media situated between the thermodynamically good solvents and precipitators for the polymer, otherwise known as a Θ-solvent. A Θ-solvent for a given polymer is a liquid media that interacts with the polymer segments with energy just sufficient to prevent the collapse of the polymer coils and precipitation of the material. Indeed, preparation of hypercrosslinked networks by crosslinking linear polystyrene chains dissolved in Θ-solvents, resulted in basically mesoporous materials [22] having an exceptionally high adsorption capacity towards β2M. Unfortunately, we failed, thus far, to prepare these materials in a beaded form of sufficient mechanical strength.

An alternative route to mesoporous hypercrosslinked networks should be the suspension copolymerization of styrene with DVB in the presence of Θ-solvents for polystyrene. This approach has been intensively examined and, indeed, resulted in useful material with an enhanced proportion of mesopores [23]. In order to create a rigid polymer of desired porosity, a sufficient portion of DVB (usually 30% or more of the co-monomer mixture) must be copolymerized with styrene in the presence of a sufficient amount of the Θ-diluent (usually 100% or more of the volume of the comonomers). Of crucial importance are the nature of the Θ-solvent, its content, and temperature. Besides cyclohexane, a classical Θ-solvent for polystyrene, mixtures of a thermodynamically good solvent (ethylene dichloride, toluene, etc.) with precipitating media (hexane, octane, isooctane, higher aliphatic alcohols, etc.), taken in an appropriate proportion, can be also applied. Microphase separation during the suspension copolymerization should take place when the major part of the comonomers has converted into polymer.

3. Properties of current adsorbing materials for β_2-microglobulin

The total volume of pores and voids of the final polymer approaches the volume of the porogen in the mixture under polymerization, provided that the network structure is rigid enough to prevent the collapse of the bead on removing the diluent after the synthesis. In our experiments, a series of copolymers was obtained with the total porosity varying between 1.0 and 1.7 cm^3/g and an apparent inner surface area

of 550–800 m^2/g. Pore size distribution, when measured through multipoint nitrogen adsorption technique, was found to be sensitive to the composition of the initial mixture under polymerization as well as the polymerization protocol. Typically, a broad pore size distribution was found with the diffuse maximum located between 15 and 25 nm. A sufficiently large portion of pores with much smaller diameters resulted in high adsorption capacity of the material towards smaller toxic molecules, which is characteristic of hypercrosslinked polystyrene materials. The typical property of the hypercrosslinked network, namely, the swelling in any liquid media was also strongly expressed. The volume of polymers increased by a factor of at least 1.3 when dry material was wetted with water and 1.5 when wetted with methanol (non-solvents for polystyrene). A further smaller increase in the volume was observed on substituting methanol for a good solvent, such as toluene. Contrary to this, typical macroporous styrene–DVB copolymers are known to maintain their volume constant, both in a dry or wetted state.

The mechanical strength of the mesoporous polymers was found to be excellent. Each bead of 0.4 mm in diameter could tolerate a load as high as 300–450 g before destruction. This is an important parameter, since crushed particles and fines released from an adsorbent of this type could embolize into the patient's circulation. The optimal bead size of the polymeric adsorbent was found to be 0.3–0.8 mm. This size prevented high backpressures at moderate to high flow-rates of viscous liquids, such as whole blood and plasma, through a cartridge packed with the polymer.

The most important property of the above mesoporous hypercrosslinked polystyrene–polydivinylbenzene type materials is their high adsorption capacity towards small proteins. Cytochrome C, which has a molecular mass of 13 000, was used as a substitute for β2M in the model adsorption experiments. From a dilute solution of cytochrome C in a neutral phosphate buffer, the polymer removed 70–98% of the protein. This corresponds to an adsorption capacity of 11–14 mg of protein per 1 ml of wet beads, or ≈30 mg per 1 g of dry polymer. In contrast to cytochrome C, adsorption of albumin from relatively concentrated solutions (35 mg/ml) was found to be moderate, about 5–7% of the initial amount.

Indeed, materials which were rated good in screening tests with cytochrome C and albumin, removed 93–98% of the β2M and <5% of albumin and other essential proteins during in vitro tests with blood or plasma from end stage renal disease (ESRD) patients (Fig. 1).

For such in vitro adsorption experiments, a Spectra/Chrom 2.5×20 cm aqueous column provided with an adjustable plunger and a water jacket is packed with 15 ml water-swollen polymer (bead size is 0.4–0.7 mm). The system is rinsed with warm saline and, to avoid dilution, the saline is replaced with 30 ml of blood (or plasma). Blood is collected from a dialysis patient in a 450-ml blood donor bag containing 63 ml of citrate–phosphate–dextrose–adenine solution that is used for blood preservation. Either whole blood or plasma are used in the dynamic adsorption experiments. A measured volume (350 ml) of blood or plasma is pooled in a saline bag and placed in a water bath at 37°C. Blood or plasma is constantly recirculated (at a rate of two bed volumes per minute) through the column. The bag is gently shaken during the 4-h test to guarantee

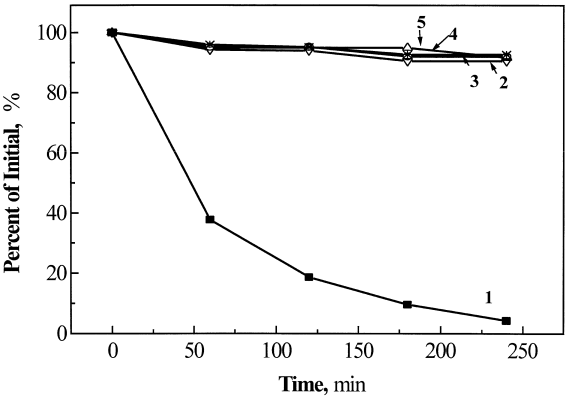

Fig. 1. Selectivity of protein sorption on the hypercrosslinked polymer in a typical dynamic in vitro experiment with plasma. The polymer to plasma ratio is 1:20 (v/v). Temperature, 37°C. Initial concentrations of the proteins are as follows: (1) β_2-microglobulin, 63.5 mg/l; (2) albumin, 3.15 g/dl; (3) prealbumin, 30 mg/dl; (4) transferrin, 217 mg/dl and (5) total proteins, 6.2 g/dl.

a homogeneous mixture. A total of ten samples are collected at definite time intervals for the subsequent determination of β2M, albumin, prealbumin, transferrin, and total protein. The analysis were performed at Spectra (California, USA).

Both the larger size of albumin molecules (6 nm in diameter, 65 000) and their higher hydrophilicity may contribute to the tendency of the mesoporous adsorbent to remove β2M (3.35 nm in diameter, 11 800) with the relatively high apparent selectivity that is shown in Fig. 1. Most important, however, is the specificity of the problem under discussion here; namely, the percent reduction of initial concentrations of corresponding proteins of interest, rather than the absolute amounts of proteins adsorbed. With the initial concentrations of albumin and β2M differing by nearly three orders of magnitude, the apparent selectivity of adsorption must be principally higher than that of dialysis. Indeed, in the course of a sorption process, the sorption sites that are attainable to albumin or other major plasma components, will soon be saturated with only a small portion of the initial protein consumed. The initial loss of major components in a dialysis process is also strongly accelerated by their high concentration gradient across the membrane. In contrast to adsorption, however, this loss will continue at the same high rate throughout the entire dialysis procedure. In other words, at a low selectivity of separation, adsorption predominantly removes the minor component of a mixture, whereas dialysis must predominantly affect the major component. This is not surprising, since dialysis is a dynamic process that is largely governed by the kinetics of diffusion, whereas in an adsorption process one steadily approaches the thermodynamic equilibrium state.

4. Biocompatibility of the polymer: in vitro and in vivo studies

During blood contact with all foreign materials, several components in the blood are activated through a variety of enzymatic and cellular processes. In the setting of chronic hemodialysis with up to 15 h weekly of blood–material interactions, the immune system, in particular, appears to be in a constantly activated, inflammatory state [24]. Some have suggested the high incidence of infection and septicemia in these patients is due, in part, to the activated immune system's inability to adequately respond to microbial pathogens. Therefore, the search for more biocompatible materials for hemodialysis and hemoperfusion is of great importance.

Previous experience with polystyrene-type adsorbent materials (Amberlite XAD-4) in clinical hemoperfusion was characterized by significant bioincompatible responses such as complement activation, neutropenia, and thrombocytopenia [25]. This was believed to be due to the hydrophobic nature of polystyrene surface. Similar problems were also noted with activated carbon adsorbents. The biocompatibility of these materials was improved by applying thin surface coatings of hydroxyethyl methacrylate, methyl cellulose, or albumin with dip or spray methods. The major drawback of these surface coatings was that they tended to reduce the efficiency of adsorption.

It has been noted earlier [10] that hypercrosslinked polystyrene does not adsorb proteins to the same extent as conventional polystyrene or macroporous polystyrene. Most probably, the openwork hypercrosslinked molecular construction does not expose any dense hydrophobic surface to the proteins. Indeed, our hypercrosslinked mesoporous materials, without any additional modification of the surface, were found to be sufficiently biocompatible and not cause any early coagulation effect in a standard plasma recalcification test. Still, in order to further enhance the biocompatibility of the polymer with blood, the outer surface of polymer beads as well as that of larger pores was subjected to chemical modification.

It had been previously recognized that the consumption of vinyl groups of divinylbenzene in its polymers and copolymers is never complete. Up to 30% of DVB involved in polymerization fails to function as a crosslinking agent and retains one of its vinyl groups intact. By analogy with hypercrosslinked polystyrene, where the remaining pendant chloromethyl groups largely concentrate in the surface of the beads and their large pores [10], it is logical to assume that the pendant vinyl groups of

the DVB polymers also concentrate on the surface. There, they fail to find a partner for the addition reaction during the polymerization process. It is convenient to use these surface exposed vinyl groups to make the surface more hydrophilic and biocompatible. We developed several simple surface modification procedures to accomplish this [26]. One approach is based on the oxidation of the vinyl groups to epoxy groups that can then be hydrolyzed to diol functions or be used for the addition of such hydrophilic species as ethanolamine, serine, aspartic acid, ethylene glycol, polyethylene glycol, etc. Another simple approach to create blood compatible materials is the grafting of long flexible hydrophilic polymer chains by radical graft-polymerization of such monomers as 2-hydroxyethyl methacrylate, acrylamide, N-vinylpyrrolidone to the pendant surface exposed vinyl groups. It is difficult to follow any of the above chemical transformation by conventional analytical techniques, unless large amounts of functional polymers are grafted onto the surface. Nevertheless, the success of the hydrophilization is always evident from the behavior of the dry polymer with respect to water. Whereas the untreated dry porous material is hydrophobic and remains floated on the surface of water, the modified material is hydrophilic. Once put into contact with water, the polymer beads easily sink. These methods of surface modification provide the necessary biocompatibility without compromising the adsorption kinetics.

The modified polymer beads passed all of the standard battery of biocompatibility tests required by the International Organization for Standardization guidelines (ISO 10993). The tests have been performed at North American Science Associates (NAMSA, Northwood, OH, USA). They included in vitro coagulation tests (plasma recalcification time), hemolysis study (extraction method), cytotoxicity study using the ISO elution method, etc. In in vivo experiments, extracts of the polymer beads did not elicit pyrogenic, irritation or sensitization reactions in laboratory animals (acute systemic toxicity study in the mouse, acute intracutaneous reactivity study in the rabbit, rabbit pyrogen study).

As further evidence of the modified polymer's excellent biocompatibility, in vivo trials incorporating the polymer into a hemoperfusion device were conducted at the University of California at Davis. A polycarbonate cartridge containing 100 ml of the polymer was steam autoclaved at 120°C for 45 min and flushed with 1 l of sterile saline prior to use. Several times, two healthy canines underwent 5 h of hemoperfusion at a flow-rate of 200 ml/min. No adverse effects such as fever or hypotension were noted. Temperature, blood pressure, mixed venous oxygen saturation, and hematocrit were continuously monitored during the procedure and all remained unchanged throughout the procedure.

Neutropenia and thrombocytopenia are commonly observed phenomena during extracorporeal blood circulation and are considered sensitive markers of biocompatibility. The graphs of white blood cell and platelet counts show a characteristic reduction at 30 min (Fig. 2), which is usually attributed to activation of the alternative pathway of complement. To generate data for comparison, the same canines were also subjected to 5 h of hemodialysis under identical conditions with a modified cellulose dialyzer (data not shown). In both the dialyzer and hemoperfusion experiments, white blood cell counts quickly return to normal and remain unchanged through the remainder of the procedure. Interestingly, platelet counts also returned to normal in the hemoperfusion group but remained by about 20% lower in the dialyzer group. This would suggest superior biocompatibility of the modified adsorbent beads. Future experiments will explore the biocompatibility of this polymer at the cellular and molecular levels by measuring

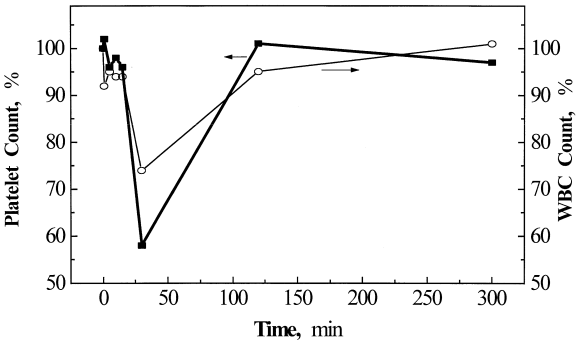

Fig. 2. Dynamics of platelet counts and white blood cell counts during a typical in vivo hemoperfusion experiment on dogs (weighing 25 kg). The cartridge incorporated 100 ml of the hypercrosslinked polystyrene that was coated with 1.5% poly(N-vinylpyrrolidone). Flow-rate, 50–100 ml/min.

Table 1
Blood chemistry during a typical canine hemoperfusion experiment

Time (min)	Cholesterol (mg/dl)	CO_2 (mEq./l)	Triglycerides (mg/dl)	Alk. phos. (I.U./l)	Mg (mg/dl)	Creatinine (mg/dl)	BUN (mg/dl)	Glucose (mg/dl)	PO_4 (mg/dl)	Ca (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Na (mEq./l)	K (mEq./l)	Cl (mEq./l)
0	134	20.6	227	22	1.2	1	17.6	90	3.2	8.6	4.6	2.4	151	3.7	122
30	137	21.0	38	22	1.2	1	16.1	111	3.2	8.9	4.7	2.5	151	3.9	122
120	134	20.0	47	21	1.3	0.9	14.5	103	3.7	9.2	4.7	2.4	152	4.0	123
300	132	19.4	47	25	1.3	0.9	10.2	107	4	9.2	4.6	2.4	148	3.9	120

specific markers of white blood cell and platelet activation such as cytokines and β-thromboglobulin.

According to the neutral nature of the polymer, no significant change in the concentration of ions was observed in the blood of the dogs during the hemoperfusion procedure. A typical example of monitoring blood chemistry in a 5-h long procedure is presented in Table 1. Table 2 shows three additional typical examples of blood chemistry before and after the hemoperfusion experiments. Interestingly, blood urea nitrogen (BUN) does gradually decrease (Table 1) to a noticeable extent during the perfusion. It may well be that our polymer is adsorbing some urea, though the latter is a very hydrophilic and small molecule. Other major blood components examined in these tests do not change significantly, with the exception of triglycerides. Adsorption of lipoproteins on surface-hydrophilized polystyrene beads remains to be examined in more detail. An alternative explanation for the drop of triglycerides could be that heparin activates enzyme lipase in the blood that degrades lipoprotein species. Since similar reduction of triglycerides was also observed in experiments with an empty cartridge as well as in standard dialysis sessions of the same dogs, the phenomenon does not appear to be a polymer-related issue.

Finally, it should be noted that β_2-microglobulin is not characteristic of blood of healthy dogs. Therefore, testing the efficiency of removing this target protein was not possible in the in vivo canine experiments.

5. Conclusions and further perspectives for hemoperfusion

For many decades, hemodialysis remains the most effective method of supporting patients with renal failure. There are over 300 000 patients in the US with chronic kidney failure and nearly 1 million worldwide. This population rapidly grows in highly developed countries where renal failure patients regularly receive hemodialysis treatment. The cost associated with kidney failure is far from trivial. In the US alone last year, over 15 billion dollars was spent on patients with chronic kidney failure. The cost of the dialysis treatment represents only 30% of these expenditures. The remainder pays for the morbidity related to chronic kidney failure, problems such as dialysis-related amyloidosis, infections, nerve dysfunction, etc. The shift toward the use of synthetic high-flux membranes with superior biocompatibility and improved removal of middle

Table 2
Blood chemistries before and after hemoperfusion procedures (three typical examples)

	Na (mEq./l)	K (mEq./l)	Cl (mEq./l)	CO_2 (mEq./l)	Ca (mg/dl)	$P0_4$ (mg/dl)	Mg (mg/dl)	Trigly. (mg/dl)	Chol. (mg/dl)	Creat. (mg/dl)	BUN (mg/dl)	Glu. (mg/dl)	Prot. (g/dl)	Alb. (g/dl)	Glob. (g/dl)	Ptase. (I.U./l)
Pre	150	4.8	118	21.7	10.2	3.1	1.7	70	164	1.1	19.8	109	6	3.3	2.7	50
Post	150	3.6	123	19.9	9.5	4	1.1	36	129	0.8	10.3	110	4.9	2.6	2.3	36
Pre	151	4.3	117	23	10.2	3	1.8	71	161	1.1	17.4	111	5.7	3.4	2.3	28
Post	148	4.1	117	22.4	10.1	4.5	1.5	44	141	0.9	14.1	99	5	2.7	2.3	25
Pre	150.5	4.55	117.5	22.35	10.2	3.05	1.75	70.5	162.5	1.1	18.6	110	5.85	3.35	2.5	39
Post	149	3.85	120	21.15	9.8	4.25	1.3	40	135	0.85	12.2	104.5	4.95	2.65	2.3	30.5

molecule toxins has not solved these problems. Most probably, providing modern expensive hemodialysis machines with additional disposable cost-efficient hemoperfusion cartridges could contribute much to reducing the costs and improving the quality of living with kidney failure.

The adsorbent polymer presented here possesses several unique and valuable properties. Its excellent biocompatibility was exhibited in both in vitro and in vivo testing. The hypercrosslinked mesoporous structure of the polymer allows for the efficient and dramatic elimination of minor proteins in the middle-molecular-mass range, like β2M, while preserving larger important proteins, such as albumin. Most intriguing is the prospect of using adsorption for the elimination of additional uremic toxins. Recent studies have identified a number of other toxic proteins, larger in size than β2M, which are believed to relate to malnutrition of renal failure patients, affect their immune system or cause cardiovascular problems. In addition, there are several small toxic molecules, like *p*-cresol or hippuric acid, which strongly bind to plasma proteins and escape elimination through dialysis [27]. A direct contact of plasma components with the hypercrosslinked polystyrene adsorbent may facilitate the dissociation of the above complexes and binding of these organic toxins in the hydrophobic micropores of the adsorbent. Similarly, the behavior of lipoproteins in contact with the polymer deserves attention, considering possibilities for conformational rearrangement of the former with a significant change in their lipophilicity.

Future studies will focus on the efficacy of combining the adsorption device with the conventional hemodialysis treatment, biocompatibility studies at the cellular and molecular level, and the elimination of additional uremic toxins.

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