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Macroporous poly(vinyl alcohol) microspheres bearing phosphate groups as a new adsorbent for low-density lipoprotein apheresis

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Abstract

A new low-density lipoprotein (LDL) adsorbent with phosphate groups as the ligand was prepared in this study. Macroporous poly(vinyl acetate-co-triallyl isocyanurate) microspheres were prepared using a free-radical suspension polymerization method. A hydrolysis reaction in sodium hydroxide/methanol changed the materials into poly(vinyl alcohol) (PVA) microspheres. Further reaction with phosphorus oxychloride in anhydrous DMF led to the LDL adsorbent PVA-phosphate microspheres. The preparation conditions such as reaction time, temperature and the amount of phosphorus oxychloride were optimized. The adsorption of plasma lipoproteins was examined by *in vitro* adsorption assays. The influence of adsorption time, plasma volume and ionic strength on the adsorption capacity was investigated. The circulation adsorption showed that the pathogenic lipoproteins in the plasma such as total cholesterol (TC), LDL and triglyceride (TG) could be removed markedly, in which the removal percentages were 42.9%, 45.0% and 44.74%, respectively. However, the reduction of high-density lipoprotein (HDL) and other normal plasma components was very slight. For *in vivo* experiment, rabbits were fed with high-cholesterol food to develop a hyperlipidemia model and treated by extracorporeal blood perfusion using the PVA-phosphate columns. Eight hyperlipidemia rabbits were treated with the PVA-phosphate adsorbent, and the removal of TC, LDL and TG was 45.03 ± 6.64%, 48.97 ± 9.92% and 35.42 ± 14.17%, respectively. The sterilization and storage tests showed that the adsorbent was chemically and functionally stable. It could be easily sterilized by a common method and stored for months without loss of adsorption capacity. Therefore, this new PVA-phosphate-based LDL adsorbent may have potential for application in LDL apheresis.

1. Introduction

Low-density lipoprotein (LDL) is the major cholesterol carrier in blood. An elevated level of LDL has been widely recognized as the major factor for the development of atherosclerosis and coronary heart disease (CHD) [1, 2]. Reduction of LDL concentration in blood can prevent or delay the progression of atherosclerosis and CHD [3]. Although diet and lipid-lowering drugs are the common methods to control the LDL level in clinical treatment [4, 5], extracorporeal elimination of LDL provides an effective way for familial hypercholesterolemia patients and those who cannot be sufficiently treated by diet and drugs. To date, several LDL

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apheresis procedures are available for clinical use: nonspecific plasma exchange [6], cascade or double filtration [7], heparin-induced extracorporeal LDL precipitation (HELP) [8] and LDL hemoperfusion using specific adsorbents [9–11]. Among these procedures, LDL hemoperfusion techniques are widely applied for safety and simple operation. The Liposorber system (Kaneka, Japan) is the most popular LDL adsorbent at present, which has been used since 1987. The adsorbent consisting of porous cellulose beads coupled with dextran sulfate can effectively reduce plasma LDL concentrations [10, 12–14]. The earlier Liposorber system could only be used for plasma apheresis due to its poor biocompatibility. The separation of plasma from whole blood made the extracorporeal circuit more complicated. The new LDL apheresis system—Liposorber D—was developed by Kaneka Corporation in 2003 based on the technology of the Liposorber LA-15 system, in which negative charged dextran sulfate was covalently bound to cellulose beads. The adsorbent could effectively reduce atherogenic lipids and lipoproteins from whole blood without biocompatibility problems [15, 16]. Another whole blood perfusion system—DALI (Fresenius, Germany)—was developed and applied clinically since 1996 [11, 17]. The adsorber consists of modified polyacrylate as affinity ligands on polyacrylamide beads. It was reported to be good in blood compatibility and could be used for whole blood treatment without the need of plasma separation [18, 19]. Clinical studies have proved that the DALI system was effective, easy to handle and shorter in treatment duration than other products [20, 21].

The success in development of Kaneka Liposorber D and Fresenius DALI indicates that the removal of LDL is mainly based on electric binding between the negatively charged dextran sulfate (or polyacrylate) and the positively charged LDL. Besides these two negative charged groups, the phosphate group is another candidate that could be used as the ligand for preparation of the LDL adsorbent. Theoretically, the charge strength of the phosphate group is between sulfate and acrylate. So, we hypothesize that the phosphate group should have the ability to adsorb LDL exactly following the principle of sulfate and acrylate groups. In addition, since phosphates are common in body fluids, an adsorbent bearing phosphate groups may have even better biocompatibility. In the present study, a new LDL adsorbent was developed by introducing negatively charged phosphate groups to macroporous poly(vinyl alcohol) (PVA) microspheres. The preparation conditions, *in vitro* adsorption, *in vivo* treatment of a rabbit hyperlipidemia model and the stability of the adsorbent were investigated.

2. Materials and methods

2.1. Materials

An adsorption column (polycarbonate, inner diameter 22 mm; length 44 mm) was manufactured by Tianjin Plastic Research Institute, China. Plasma from hyperlipidemic patients was kindly supplied by General Hospital of Tianjin Medical University. Vinyl acetate, triallyl isocyanurate (TAIC), ethyl acetate, n-heptane, 2, 2′-azobisisobutyronitrile (AIBN), *N*,*N*′-dimethylformamide (DMF) and phosphorus oxychloride were purchased from Guangfu Fine Chemical Research Institute (Tianjin, China). Rabbits were purchased from the Laboratory Animal Center of The Academy of Military Medical Sciences (Beijing, China). The animal studies were performed in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals (Tianjin, revised in June 2004).

2.2. Preparation of PVA microspheres

Crosslinked macroporous PVA microspheres were prepared using a free-radical suspension polymerization method. Vinyl acetate, TAIC, mixture of ethyl acetate and n-heptane and AIBN were used as monomer, crosslinker, porogen and initiator, respectively. A 1000 mL flask was charged with a homogeneous liquid mixture containing 67.6 mL of vinyl acetate, 27 g of TAIC, 45 mL of ethyl acetate, 45 mL of n-heptane and 2.28 g of AIBN. The temperature was raised to 40 °C with stirring, and 500 mL of an aqueous solution containing 1% (w/v) of polyvinyl alcohol (PVA, 88% hydrolyzed, M_n = 14 900), 1.5% (w/v) of disodium hydrogen phosphate dodecahydrate and 0.05% (w/v) of sodium dihydrogen phosphate dehydrate were added to the flask. The mixture was stirred with proper speed and the temperature was raised to 65 °C and sustained for 3 h. Then, the flask was heated to 75 °C and kept at this temperature for another 3 h for complete polymerization. The resultant copolymer microspheres of poly(vinyl acetate-co-triallyl isocyanurate) were filtered, washed with distilled water, extracted with acetone to remove the unreacted reagents and finally dried at 45 °C under vacuum. The PVA microspheres were prepared by alcoholysis which was carried out at 40 °C for 48 h in 1.5 L of 3% (wt) solution of sodium hydroxide/methanol. The PVA microspheres were filtered, washed with distilled water and dried at 45 °C under vacuum. Figure 1 shows the preparation scheme of PVA microspheres.

2.3. Preparation of the PVA-phosphate adsorbent

The adsorbent was prepared by introducing the phosphate groups to the PVA microspheres. Ten grams of dried PVA microspheres and 100 mL of anhydrous DMF were added into a 250 mL three-neck flask. The temperature was raised to 120 °C and kept for 30 min. Thirty milliliters of phosphorus oxychloride was slowly added to the reaction system with stirring, and the temperature was retained at 120 °C for 3 h. After the temperature was slowly decreased to 0 °C with ice water, NaOH solution (10%wt) was added to the reaction system dropwise until the phosphorus oxychloride was completely hydrolyzed. The adsorbent microspheres were filtered and thoroughly washed with distilled water.

2.4. Characterization of the PVA-phosphate adsorbent

The content of the hydroxyl groups on PVA microspheres was determined by an acetic anhydride/pyridine titration method, as described previously [22]. The FTIR spectra of poly(vinyl acetate-co-triallyl isocyanurate) and PVA microspheres were

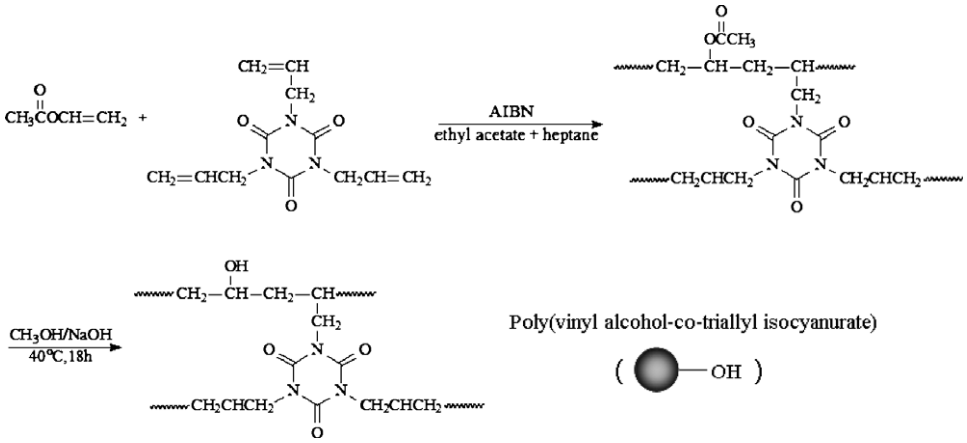

Figure 1. Preparation scheme of PVA microspheres.

measured on a 560ESP FTIR spectrometer (Thermo Nicolet). The surface morphology and the size of the PVA microspheres were observed by scanning electron microscopy (SEM, X-650, Hitachi). The pore size of the microspheres was estimated according to the SEM images. The specific surface area of the microspheres was measured on BET nitrogen adsorption apparatus (Micromeritics, ASAP 2010). The skeleton density, apparent density, pore volume and porosity were measured following the methods previously described [23, 24]. The mechanical strength of PVA microspheres was tested according to the literature [25].

2.5. In vitro static adsorption

The adsorption performance of the adsorbents was evaluated by a static adsorption method. Before the test, the adsorbent was equilibrated with normal saline for several hours, and then 3 mL of hyperlipidemic plasma was mixed with 1 mL of the adsorbent in a polypropylene tube and incubated at 37 °C for 3 h on a shaker. The supernatant plasma was collected, and the concentration of total cholesterol (TC), LDL, triglyceride (TG) and high-density lipoprotein (HDL) was examined using test kits (Zhongsheng Beikong Bio-technology and Science Inc., Beijing, China) following the instruction of manufacturers. All the adsorption tests were performed in triplicate.

2.6. Circulation adsorption

A circulation system was set up which was similar to the system presented in the literature [26]. Fifteen millimeters of adsorbent was packed in a polycarbonate adsorption column and primed with normal saline. Hyperlipidemic plasma (75 mL) in a reservoir (placed in water bath, 37 °C) was infused through the adsorbent column by a peristaltic pump. The blood flow rate was 1.5 mL min^{-1} which was controlled by the pump, and the circulation was performed for 3 h. The concentration of plasma components was examined with an automatic biochemical analyzer (7170, Hitachi). The adsorption tests were performed in triplicate.

2.7. Stability tests

The stability of the adsorbent was examined by sterilization and storage tests. Simply, the adsorbent was placed in an autoclave; after sterilization for 30 min at 121 °C, it was washed sufficiently with normal saline and the adsorption capacity was determined. In addition, the adsorbent was stored in normal saline at 4 °C, and the adsorption capacity was examined at regular intervals.

2.8. Hemoperfusion

The hyperlipidemia rabbit model was developed according to the literature [27]. Eleven rabbits were divided into two groups, experimental group ($n = 8$) and control group ($n = 3$). Whole blood perfusion was performed using regular circulation pump. The adsorption column containing 28 mL of PVA-phosphate adsorbent was first rinsed with 250 mL of rinsing solution (237.5 mL of physiological saline, 6.25 mL of acid citrate dextrose solution and 6.25 mL of 8.4% sodium bicarbonate solution), and then primed with 250 mL of priming solution (10 IU mL^{-1} of heparin in saline). Blood was drawn from the carotid artery and returned to the carotid vein at a flow rate of 5 mL min^{-1}. Heparin was used as the start anticoagulant at a dose of 150 IU kg^{-1} body weight. During the treatment, anticoagulation was achieved by continuous infusion of the acid citrate dextrose solution at the rate of 10 mL h^{-1}. Blood samples were taken from the arterial bloodline, and the lipoproteins were determined with an automatic biochemical analyzer (7170, Hitachi). The control animals were treated with the same procedures but using empty columns.

3. Results

3.1. Preparation and characterization of PVA microspheres

Poly(vinyl acetate-co-triallyl isocyanurate) microspheres were transformed into PVA microspheres through the alcoholysis reaction. FTIR spectroscopy was used to monitor the alcoholysis procedure. The appearance of strong stretching vibration adsorption at 3422 cm^{-1} and the disappearance of stretching vibration adsorption at 1739 cm^{-1} and 1243 cm^{-1} indicated that the ester groups in vinyl acetate were sufficiently alcoholyzed into hydroxyl groups. The content of hydroxyl groups in the PVA microspheres was 1.95 mmol g^{-1}.

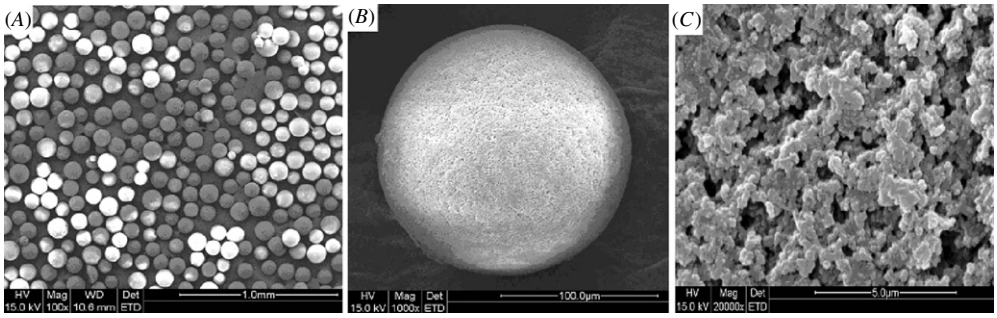

Figure 2. SEM images of PVA microspheres. (*A*) 100×; (*B*) 1000×; (*C*) 20 000×.

The surface morphology of PVA microspheres was examined by SEM. As shown in figure 2(*A*), the PVA particles are typical spheres with narrow size distribution ranging from 100 to 150 μm. Rough surface, macroporous and bulk structure can be clearly seen in figures 2(*B*) and (*C*). The pore size was 200–1000 nm as estimated from the SEM images. The specific surface area was 36.49 $m^2\ g^{-1}$. The skeleton density and apparent density were 1.63 g mL^{-1} and 0.42 g mL^{-1}, respectively. The pore volume was 1.74 mL g^{-1} and the porosity was 73.9%. The breakage of the microspheres was less than 0.83%, which indicates that the microspheres have good mechanical strength.

3.2. Preparation of the PVA-phosphate adsorbent

The content of phosphate groups introduced into the PVA microspheres depends on the preparation conditions, such as reaction time, temperature and the amount of phosphorus oxychloride applied. As shown in figure 3, the adsorption of TC, LDL and TG increased with the increase in reaction time, reaction temperature and $POCl_3$/microsphere volume ratios. We chose 120 °C, 120 min and $V(POCl_3)/V(\text{microsphere}) = 2$ as the optimal conditions. Preparation at higher temperature and longer reaction time may reduce the mechanical strength of the particles.

3.3. In vitro static adsorption

Figure 4(*A*) illustrates the dynamic adsorption of the PVA-phosphate adsorbent. The adsorption of TC, LDL and TG increased gradually and reached equilibrium at about 3 h, while the adsorption of HDL reached equilibrium quickly within 15 min. Figure 4(*B*) shows the effect of the plasma volume on the adsorption capacity. The adsorption of four lipoproteins increased with the increase in the plasma volume. The maximum adsorption capacities for TC, LDL, TG and HDL were 2.82, 1.70, 2.25 and 0.61 mg mL^{-1}, respectively.

The effect of ionic strength on lipoprotein adsorption is shown in figure 4(*C*). The ionic strength in the plasma was adjusted with NaCl solutions of different concentrations. With the increase in ionic strength from 0 to 1.0 mol L^{-1}, the adsorption of TC, LDL and TG decreased by 16.0%, 25.5% and 20.2% respectively. But the adsorption of HDL was not affected by the ionic strength. The effect of ionic strength on the adsorption may be due to the ionic atmosphere effect [28]. The existence of ionic atmosphere may weaken or destroy the electric interactions between the positively charged lipoproteins and the negatively charged phosphate groups. Figure 5(*A*) displays the relationship curve between the equilibrium adsorption of LDL and the initial LDL concentration in the plasma. It can be seen that the adsorption capacity of LDL increased from 0.44 mg mL^{-1} to 2.09 mg mL^{-1} when the initial LDL concentration increased from 15 mg dL^{-1} to 180 mg dL^{-1}.

The affinity adsorption procedure can be described by two theoretical isotherm models: Langmuir model and Freundlich model. The two models can simply fit the relationship between the concentration of adsorbate and its amount of adsorbed to the solid phase when two phases are at equilibrium. The Freundlich model usually fits the experimental data well in the aqueous solution adsorption system [29], while the Langmuir model is used to describe the adsorption phenomenon in the human plasma system [30]. With the typical Langmuir model, the adsorption of LDL in this study can be described by the following Langmuir isotherm equation:

$$\frac{C_{\text{eq}}}{Q} = \frac{K_d}{Q_{\max}} + \frac{1}{Q_{\max}} \times C$$

where Q (mg mL^{-1}) stands for the amount of LDL adsorbed on the adsorbent at equilibrium, $Q_{\max}$ (mg mL^{-1}) for the maximum value of Q, C (mg dL^{-1}) for the equilibrium concentration of LDL in plasma and K_d is a constant. As seen from the straight-line plot of C_{eq}/Q versus C by linear regression, the values of K_d and $Q_{\max}$ in our adsorption system were 93.47 mg dL^{-1} and 3.18 mg mL^{-1}, respectively. The correlation coefficient was as high as 0.999 75. These data indicate that the adsorption of LDL from the human plasma by the PVA-phosphate adsorbent fits the Langmuir isotherm well and implies a monolayer adsorption.

3.4. In vitro flow adsorption

In flow adsorption tests, the concentration of plasma lipoproteins decreased over circulation time (figure 6). After 3 h, the removal of TC, LDL, TG and HDL was 42.9%, 48.1%, 42.0% and 24.1%, respectively. Non-specific adsorption was also investigated by measuring the content of other major components in the plasma. As shown in table 1, the pathogenic lipoprotein(a) was reduced by about 50%. However, the reduction of all other normal plasma components was less than 20%. This suggests that the PVA-phosphate adsorbent is biocompatible and may not have adverse impact on plasma components.

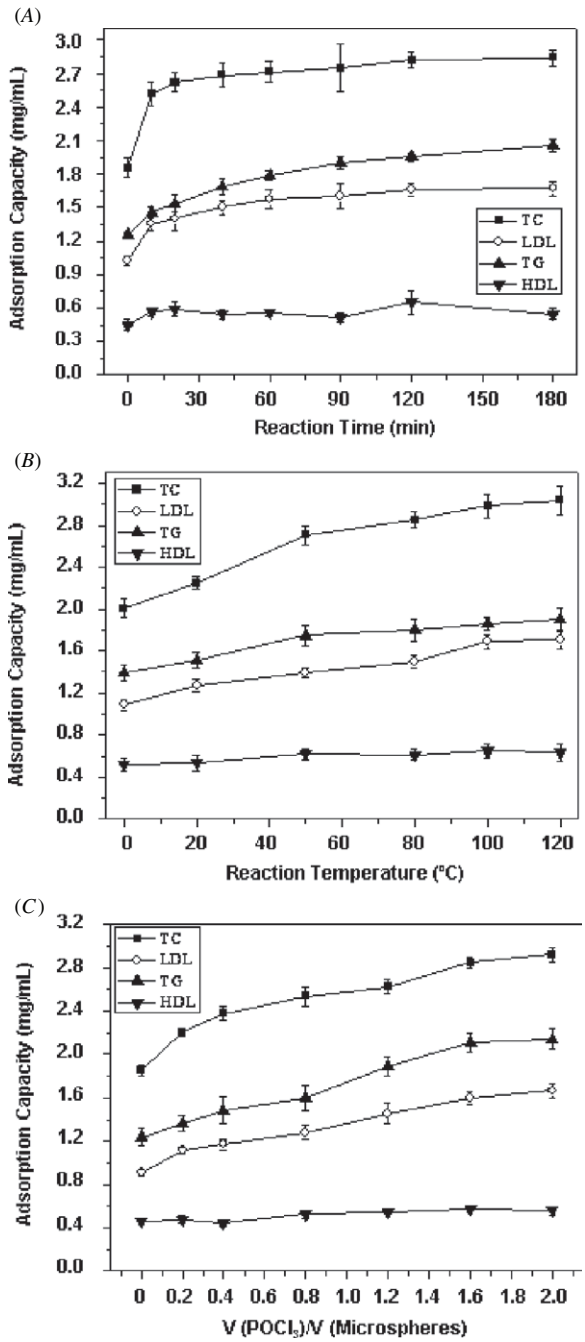

Figure 3. Effect of preparation conditions on lipoprotein adsorption ($n = 3$). (*A*) Effect of reaction time: 120 °C, $V(POCl_3)/V$(microspheres) = 2. (*B*) Effect of temperature: 180 min, $V(POCl_3)/V$(microspheres) = 2. (*C*) Effect of $V(POCl_3)/V$(microspheres) ratio: 120 °C, 180 min.

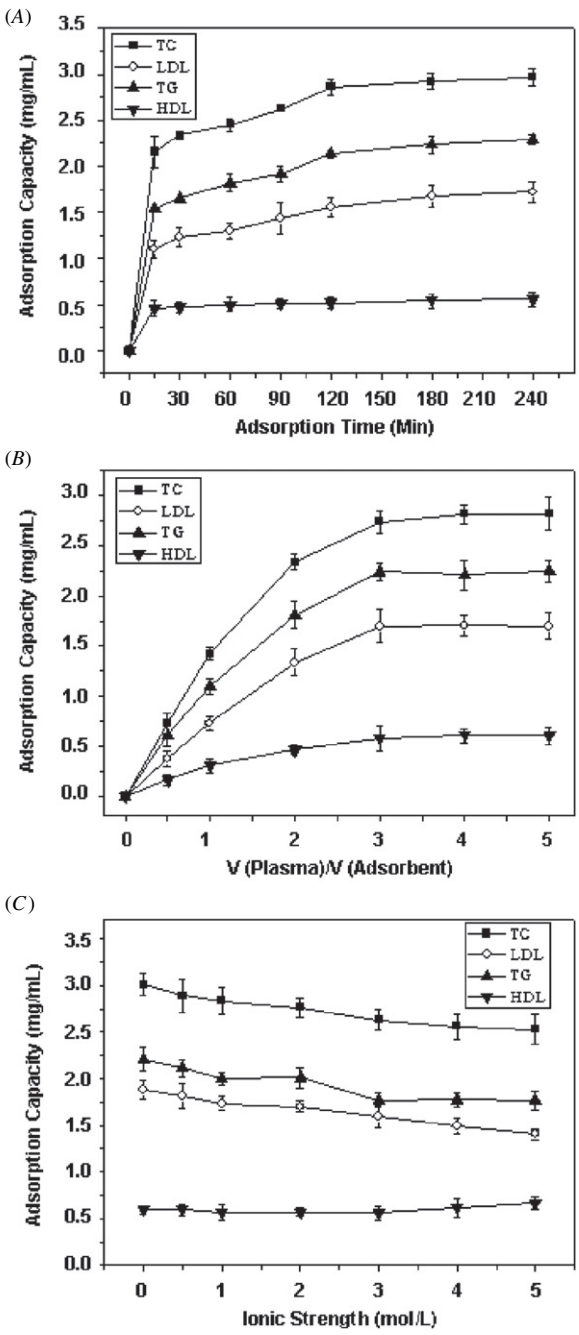

Figure 4. *In vitro* adsorption of plasma lipoproteins ($n = 3$). (*A*) Effect of adsorption time: 37 °C, V(plasma)/V(adsorbent) = 3, no addition of ions. (*B*) Effect of V(plasma)/V(adsorbent) ratio: 37 °C, 180 min; no addition of ions. (*C*) Effect of ionic strength: 37 °C; V(plasma)/V(adsorbent) = 3, 180 min.

3.5. Stability

As shown in figure 7(*A*), the loss of adsorption for TC, LDL, TG and HDL caused by the autoclaving was as little as 6.77%, 5.42%, 5.77% and 5.09%, respectively. In other words, the remaining adsorption capacity was over 93%. After storage in normal saline at 4 °C for 24 weeks, the loss of adsorption capacity for TC, LDL, TG and HDL was only 7.36%, 5.36%, 12.87% and 1.85%, respectively (figure 7(*B*)).

3.6. In vivo adsorption

The removal of lipoproteins from the blood of hyperlipidemia rabbits by the PVA-phosphate adsorbent is shown in figure 8. In the treatment group ($n = 8$), TC, LDL and TG were significantly reduced from the initial concentration 256.67 ± 66.38 mg dL^{-1}, 213.40 ± 56.13 mg dL^{-1} and 91.81 ± 32.57 mg dL^{-1} to 141.10 ± 32.25 mg dL^{-1}, 108.89 ± 20.72 mg dL^{-1} and 59.29 ± 25.41 mg dL^{-1} within 2 h. The removal of TC, LDL and TG was 45.03 ± 6.64%, 48.97 ± 9.92%, and 35.42 ± 14.17%, respectively, whereas HDL,

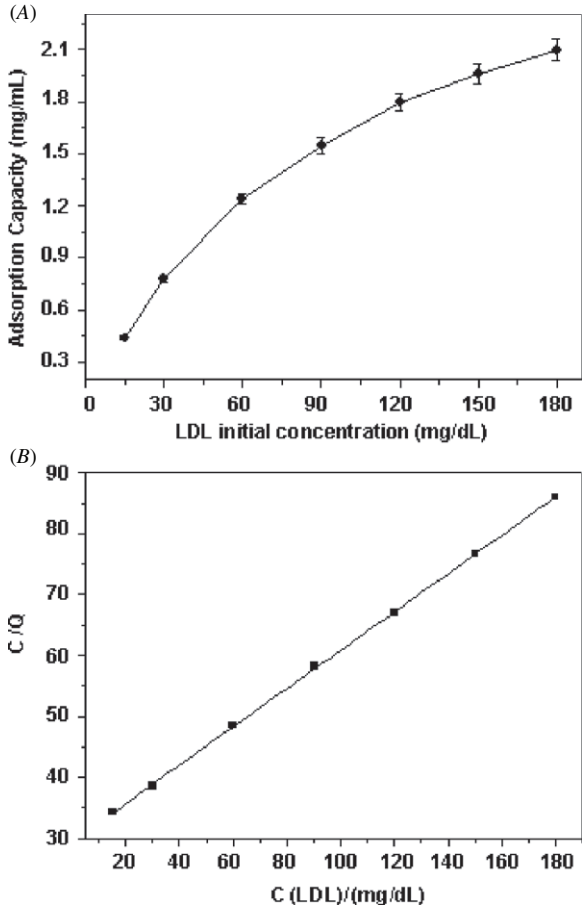

Figure 5. Isothermal adsorption of LDL ($n = 3$). (A) Effect of LDL initial concentration on LDL adsorption: 37 °C, V(plasma)/V(adsorbent) = 3, 180 min. (B) The Langmuir adsorption isotherm of LDL on a PVA-phosphate adsorbent: 37 °C, V(plasma)/V(adsorbent) = 3, 180 min.

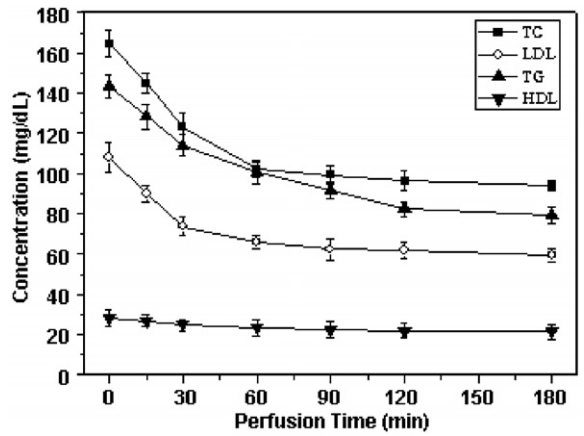

Figure 6. Effect of perfusion time on lipoprotein reduction in flow adsorption: 37 °C; V(plasma)/V(adsorbent) = 5.

the helpful concentration, was slightly decreased from 26.05 ± 14.59 mg dL^{-1} to 21.01 ± 14.27 mg dL^{-1} (removal 19.33 ± 5.10%). In contrast, the reduction of the four lipoproteins was below 5% in the control group ($n = 3$) (data not shown). These data indicate that the PVA-phosphate adsorbent can selectively remove the elevated TC, LDL and TG from the plasma.

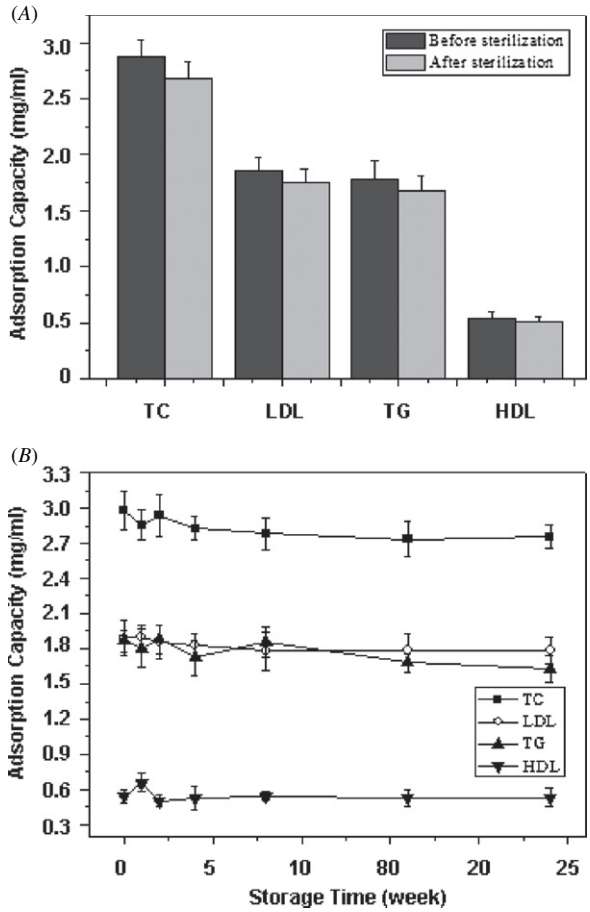

Figure 7. Effect of autoclaving (A) and storage (B) on lipoprotein adsorption: 37 °C, V(plasma)/V(adsorbent) = 3, 180 min.

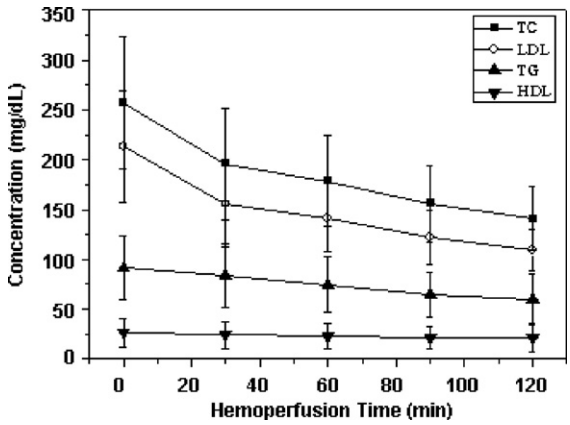

Figure 8. Reduction in lipoproteins from the blood of hyperlipidemia rabbits in the animal experiment ($n = 8$).

4. Discussion

The present paper describes a new adsorbent for the removal of LDL from human plasma. The adsorbent was based on PVA microspheres as a supporting matrix. For any adsorbents, the adsorption capacity is usually determined by the pore size and the surface area of the matrix. Human LDL particles are essentially spherical and approximately 25 nm in diameter [31]. The pore diameter of PVA microspheres prepared and used in this study is between 200 and 1000 nm. The LDL

Table 1. Effect of plasma perfusion on the plasma components

Parameters	Concentration before	Concentration after	Reduction (%)
Lipoprotein(a) (mg dL^{-1})	2.87 ± 0.23	1.40 ± 0.50	50.94 ± 17.17
Total protein (g L^{-1})	61.33 ± 6.43	56.33 ± 2.08	7.67 ± 6.98
Albumin (g L^{-1})	28.00 ± 1.00	24.33 ± 0.58	13.02 ± 3.66
Globulin	33.33 ± 5.03	29.00 ± 5.57	13.38 ± 3.92
Alanine-aminotransferase (U L^{-1})	17.67 ± 0.58	14.33 ± 0.58	18.84 ± 2.96
Aspartate-aminotransferase (U L^{-1})	21.33 ± 5.78	20.00 ± 1.00	6.02 ± 3.77
Alkaline phosphatase (U L^{-1})	106.00 ± 27.18	99.00 ± 25.51	6.53 ± 3.29
γ-Glutamyltransferase (U L^{-1})	8.33 ± 1.53	7.67 ± 1.53	8.09 ± 7.33
Total bilirubin (μmol L^{-1})	0.90 ± 0.10	0.73 ± 0.06	18.24 ± 5.09
Urea (mmol L^{-1})	5.53 ± 0.06	4.73 ± 0.12	14.46 ± 1.82
Creatinine (μmol L^{-1})	73.67 ± 1.15	69.33 ± 1.53	5.86 ± 2.76
Calcium (mmol L^{-1})	2.97 ± 0.16	2.88 ± 0.15	3.02 ± 0.46
Potassium (mmol L^{-1})	2.92 ± 0.35	2.87 ± 0.62	1.64 ± 0.86
Sodium (mmol L^{-1})	140.67 ± 4.93	138.00 ± 6.08	1.92 ± 1.14
Chloride (mmol L^{-1})	99.00 ± 5.57	97.33 ± 6.51	1.72 ± 1.27
Immunoglobulin A (mg dL^{-1})	184.30 ± 9.29	177.33 ± 10.41	3.82 ± 1.08
Immunoglobulin G (mg dL^{-1})	993.30 ± 26.5	958.30 ± 25.54	3.52 ± 0.03
Immunoglobulin M (mg dL^{-1})	96.00 ± 3.54	90.00 ± 2.98	6.24 ± 0.35
Complement 3 (mg dL^{-1})	101.80 ± 7.41	96.30 ± 5.67	5.37 ± 1.27
Complement 4 (mg dL^{-1})	24.70 ± 1.25	21.30 ± 1.85	13.69 ± 4.90

Temperature: 37 °C; V(plasma)/V(adsorbent) = 5; perfusion time: 180 min; no addition of ions.

particles can diffuse through the pores into the inner porous structure of the microspheres easily, and thus enhance the adsorption capacity. In addition, the large pore volume and good mechanical strength also made the PVA microspheres suitable for the LDL adsorbent supporting matrix.

The specific ligands immobilized on the supporting matrix also play an important role in LDL adsorption. Many chemical and biological molecules have been extensively studied and applied as specific LDL ligands [10, 17, 32–35]. Among these ligands, anionic dextran sulfate and polyacrylate ligands are successfully used to develop negatively charged LDL adsorbents and have been used for clinical treatment. In this paper, the phosphate group was used as an LDL ligand. Similar to the binding between LDL and dextran sulfate or polyacrylate, the positively charged apoprotein B moiety on LDL particles can interact with the negatively charged phosphate groups immobilized on PVA microspheres through an electrostatic interaction. The experimental results also proved that the PVA-phosphate adsorbent can selectively remove LDL from human plasma with a capacity similar to commercially available LDL adsorbers. Since phosphates are very common in human body fluid, such a phosphate-based LDL adsorbent *may have a better* blood compatibility than those LDL adsorbents using dextran sulfate or polyacrylate as the ligands. Although the data in table 1 have demonstrated the selectivity and safety of this LDL adsorbent, more experiments are needed to prove other advantages of this PVA-phosphate microsphere.

The PVA microspheres prepared in this study are highly crosslinked, and thus are good in mechanical strength. It allows the phosphorylation reaction to be conducted at high temperature and anhydrous conditions. Such conditions help to introduce the high content of phosphate groups to the PVA microspheres for maximum LDL adsorption. This could be an advantage of PVA microspheres over cellulose or sepharose beads which are usually not available to conduct the phosphorylation reaction under the above extreme conditions.

In the animal experiment, the removal percentage of TC, LDL, TG and HDL was 45.03 ± 6.64%, 48.97 ± 9.92%, 35.42 ± 14.17% and 19.33 ± 5.10%, respectively. These adsorption data are comparable to the reported LDL adsorbents [22, 26, 34–36] and the commercial apheresis systems, such as DALI and Liposorber D (DALI: 49.75%, 63.56%, 48.10% and 8.93%; Liposorber D: 58.65%, 68.94%, 64.29% and 18.52%, respectively) [16].

Ionic strength has an influence on the LDL adsorption, confirming that the adsorption of LDL to phosphate is through the electrostatic interaction. Pretreatment of the adsorbent with electrolyte solutions may influence the adsorption capacity by changing the ionic types of the phosphate. The priming solution which contains heparin or angelica polysaccharide for anticoagulation may also influence the binding between phosphate and LDL since heparin and angelica polysaccharide are both highly charged molecules. Although the current study only used heparin-containing saline as the priming solution, we assume that a pretreatment buffer or priming solution with well-defined components may be able to further improve the adsorption capacity.

As to the stability of the adsorbents, the adsorption capacity was slightly influenced by autoclaving and long-term storage. The high temperature and pressure during autoclaving may cause some changes of the pore structure. Further experiments are required to optimize the sterilization methods and minimize the loss of adsorption capacity.

5. Conclusions

In the present study, a new adsorbent for the removal of LDL was developed by introducing the phosphate group onto

macroporous PVA microspheres. This is the first PVA-phosphate-based LDL adsorbent. The *in vitro* adsorption results show that the PVA-phosphate adsorbent can efficiently remove TC, TG and LDL from the hyperlipidemic plasma with low non-specific adsorption. This adsorbent has the potential for whole blood perfusion in treatment of hypercholesterolemia.

Acknowledgments

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