Recombinant Protein A Immobilized on Cross-linked Cellulose Microspheres for Immunoglobulin G Adsorption from Human Plasma

Xiaodong Cao,[a,b] Biyan Zhu,[a,b] Tao Chen,[c] Xufeng Zhang,[d] and Hua Dong [a,b,*]

Cross-linked cellulose microspheres (CL-CMs) were successfully prepared using an inverse crosslinking suspension method from a cellulose solution with sodium hydroxide/urea aqueous solution as a solvent and epichlorohydrin as the crosslinker. The effects of epichlorohydrin content on the appearance and dispersity, average pore volume, moisture content, and wet real density of CL-CMs were studied. The microspheres presented a good spherical shape and porous surface structure. After activation with $NaIO_4$, the recombinant protein A was immobilized onto the surface of CL-CMs to form an immunoadsorbent. Adsorbents containing various amounts of protein A were applied to adsorb immunoglobulin G (IgG) from human plasma. The maximum IgG adsorption capacities with static adsorption and dynamic adsorption were 23 and 13 mg, respectively, per gram of CL-CMs carrying 6.8 mg of recombinant protein A. Therefore, CL-CMs immobilized with recombinant protein A have great potential for application in the field of blood purification.

Keywords: Cellulose microspheres; Inverse crosslinking suspension; Protein A; Blood purification; IgG

Contact information: a: School of Materials Science and Engendering, South China University of Technology, Guangzhou, 510641, PR China; b: Guangdong Province Key Laboratory of Biomedical Engineering, South China University of Technology, Guangzhou 510640, PR China; c: Department of Internal Medicine, Mile Hospital of Yunnan Province, Mile 652300, PR China; d: College of Chemistry and Chemical Engineering, Yunnan Normal University, Kunming 650092, PR China;
** Corresponding author: donghua@scut.edu.cn*

INTRODUCTION

Staphylococcal Protein A (SPA) is a cell wall-associated protein domain exposed on the surface of the gram-positive bacterium *Staphylococcus aureus* (Hober *et al*. 2007). SPA has significance to biotechnology and bioscience because of its specific binding to the Fc portion of immunoglobulin G (IgG) from many mammals (Jungbauer and Hahn 2004). The selectivity for IgG varies with species and subclasses (Richman *et al*. 1982). Human IgG is bound with high affinity except for IgG3, which is weakly bound (Langone 1982). Therefore, the protein A immunoadsorption column is the most commonly used for the treatment of autoimmune diseases such as systemic lupus erythematosus (Braun *et al*. 2000), myasthenia gravis (Somnier and Langvad 1989), and rheumatoid arthritis (Braun and Boseh 2000) because of the excellent properties of protein A, such as high selectivity of antibodies, easy immobilization on carriers, and high stability (Walker 2002). Meanwhile, the carrier material also plays an important role because it not only relates to immobilizing protein A, but also affects the adsorption capacity and efficiency (Shtilman 1993). The ideal carrier for protein A immobilization should possess the following features (Kundu and Roy 1979; Jaulmes *et al*. 2001): 1) high insolubility in water and

hydrophilicity; 2) steady physical and chemical properties and low non-specific adsorption; 3) high specific surface area and active groups; and 4) high mechanical strength and rigidity. Currently, commercial protein A immunoadsorbents use silica microspheres or cross-linked agarose beads as carriers. In recent years, the number of therapeutic systemic autoimmune diseases in clinical trials has increased enormously, and immunoadsorption columns based on protein A are used extensively. However, silica microspheres have poor blood compatibility and high nonspecific adsorption, while agarose beads have a mechanical intensity deficiency, and the high treatment fee means that most of the patients cannot afford it. Therefore, developing a lower-cost and higher biocompatibility material for protein A immunoadsorbent to meet the demands of clinical application is essential.

Cellulose, the most abundant natural polysaccharide, has attracted more and more attention because of its biodegradability, non-toxicity, environmental friendliness, and biocompatibility (Mohanty *et al*. 2002; Klemm *et al.* 2005; Fukuzumi *et al*. 2009). Cellulose beads were first prepared by O'Neill and Reichardt (1951) from a cellulose viscose solution and showed great features such as high hydrophilicity and mechanical strength. Since then, cellulose beads have been used in many advanced applications ranging from chromatography over solid supported synthesis and protein immobilization to the retarding of drug release (Gericke *et al.* 2013). However, cellulose viscose solution is toxic, and poisonous gases such as CS_2 and H_2S are released during its preparation process, which therefore involves an element of risk if it is applied as a biomedical material (Woodings 2001). Recently, Zhang's group developed a series of novel solvents to dissolve cellulose rapidly with LiOH or NaOH and urea or thiourea aqueous solutions at low temperatures (Cai and Zhang 2005; Mao *et al.* 2006; Luo and Zhang 2013). With alkali hydroxide/urea aqueous solutions as solvents, cellulose microspheres have been prepared and applied to various fields. Luo and Zhang (2010) prepared regenerated cellulose microspheres with different diameters in an NaOH/urea aqueous solution by the sol–gel transition method, which were used for the fractionation of polyethylene oxide. Wu *et al.* (2012) fabricated cellulose/silver nanoparticles composite microspheres from a NaOH/thiourea solution by combination of sol–gel method with hydrothermal treatment, and the Ag NPs showed high catalytic activity. Cellulose microspheres are also widely used in extracorporeal blood purification because of their high biocompatibility. Cellulose particles were activated and covalently immobilizated of anti-TNF-α antibody, which yielded a highly efficient adsorption performance for the pro-inflammatory cytokine, showing application potential in suspension-based systems for extracorporeal blood purification (Ettenauer *et al.* 2011). Kong *et al.* (2000) synthesized immunoadsorbents using epichlorohydrin to activate cellulose microspheres to immobilize DNA, and then they treated systemic lupus erythematosus through plasma perfusion. Studies on the synthesis of protein A immunoadsorbent using regenerated cellulose microspheres as a carrier for removing IgG from human plasma have rarely been reported (Cao *et al*. 2007).

In this work, we prepared cross-linked cellulose microspheres (CL-CMs) from a NaOH/urea aqueous solution using the inverse crosslinking-emulsion method and then immobilized protein A onto the CL-CMs through $NaIO_4$ activation, which was green, simple, and convenient. Static adsorption and dynamic adsorption of IgG from human plasma were studied carefully to evaluate the possibility for application in the blood purification field.

EXPERIMENTAL

Materials

Cellulose (cotton linter pulp) was provided by the Hubei Chemical Fiber Group, Ltd. (Xiangfan, China). Recombinant protein A consisted of three tandem B domains of protein A and was supplied by Guangzhou Koncen Bioscience Co., Ltd. (Guangzhou, China). A Pierce BCA protein A assay kit was purchased from Thermo Fisher Scientific (Massachusetts, USA). Epichlorohydrin (ECH) and $NaIO_4$ were purchased from the Tianjin Fuchen Chemical Reagents Factory (Tianjin, China). $NaBH_4$ was obtained from Shanghai Rich Joint Chemical Reagent Co., Ltd. (Shanghai, China). Span 80 was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Fresh-frozen human plasma was obtained from a local plasma donation center. Other chemicals of analytical grade were used as received.

Preparation of Cross-linked Cellulose Microspheres

Alkali hydroxide/urea aqueous solution was used as the solvent to dissolve cellulose according to Cai and Zhang (2005). A solution of 7 wt% NaOH/12 wt% urea/81 wt% H_2O was pre-cooled to −12 °C, and then 11 g of cellulose were added to the solvent (300 mL). The suspension was vigorously stirred at room temperature for 5 min until a transparent cellulose solution was obtained. The cellulose solution was degassed through centrifugation at 6000 rpm for 10 min at 4 °C. The cellulose microspheres were prepared using the inverse crosslinking-emulsion method. Two hundred milligrams of liquid paraffin and 10 g of span 80 were added into a three-necked, round-bottomed flask and stirred at 450 rpm for 30 min. Subsequently, 60 mL of prepared cellulose solution was dropped into the flask within 30 min. After stirring for another hour, various amounts of epichlorohydrin were added dropwise to the flask separately. The suspension was emulsified for 3 h under a constant stirring speed and then heated to 50 °C and maintained for an additional 2 h. Finally, a mixed aqueous solution of H_2SO_4 and Na_2SO_4 (Na_2SO_4: H_2SO_4: H_2O = 5 wt%: 5 wt%: 90 wt %) was added to neutralize the mixture. After centrifuging, the suspension was separated into two layers, and the lower layer was collected and washed with 20% acetone, 50% ethanol, and deionized water, subsequently. The cross-linked cellulose microspheres were obtained and coded as CL-CM1, CL-CM2, CL-CM3, and CL-CM4 corresponding to the added volume of epichlorohydrin (3%, 5%, 7%, or 8%, respectively).

Activation of Cross-linked Cellulose Microspheres

Ten grams of CL-CM2 were mixed with 50 mL of $NaIO_4$ solution with various concentrations (0.01, 0.02, 0.03, 0.04, and 0.05 M) in a conical flask. The reactor was put into the shaker and incubated at 40 °C with gentle shaking for 2 h in the dark. After washing with massive distilled water, the activated cellulose microspheres were obtained and stored at 4 °C in a refrigerator for further use.

The amount of dialdehyde groups on the activated microspheres was determined using the following method: 0.5 g of activated microspheres were treated with 50 mL of 20 g/L hydroxylamine hydrochloride-methanol solution with gentle shaking at room temperature for 10 min. Hydroxylamine hydrochloride easily reacted with dialdehyde and released H^+. Then, 0.03 M NaOH-methanol solution was used to neutralize the released H^+ during the reaction, with thymol blue as the indicator.

SPA Immobilization of Activated Cross-linked Cellulose Microspheres

Five grams of activated CL-CM2 with various activation densities were washed five times with deionized water and then treated with 5 mL of borate buffer solution and 5 mL of recombinant protein A physiological saline solution. The initial concentration of protein A buffer solution changed from 2 mg/mL to 12 mg/mL. The reaction was kept at 37 °C for 24 h, while the stirring rate was maintained at 170 rpm. After coupling, 30 g of phosphate buffered saline (PBS) containing 0.5% $NaBH_4$ was used to reduce the microspheres at the same temperature for 4 h. The concentration of protein A before and after the binding reaction was assayed by the BCA protein determination method with the Pierce BCA protein A assay kit.

Characterization of Cross-linked Cellulose Microspheres

The morphology of dried cellulose microspheres (CL-CM2) was observed with a NOVA NANOSEM 430 environmental scanning electron microscope (Holland) with an accelerating voltage of 20 kV.

The wet real density and moisture content of CL-CMs were characterized using the following methods: The wet real density of CL-CMs was measured using the water substitution method with a 5-mL pycnometer, using deionized water for steeping. Moisture content could reflect the hydrophilicity of the medium, which was measured by drying the wet microspheres in a vacuum oven at 120 °C to a constant weight. The wet real density (ρ_p), moisture content (ω), and average pore volume (V_p) were calculated according to Eqs. 1 through 3,

$$\rho_p = \frac{M_1 \times \rho_w}{M_1 + M_2 - M_3} \quad (1)$$

$$\omega = \frac{m_2 - m_3}{m_2 - m_1} \times 100\% \quad (2)$$

and

$$V_p = \frac{\omega}{(1-\omega)\cdot\rho_w} \times 100\% \quad (3)$$

where M_1 is the mass of the wet microspheres and M_2 and M_3 are the mass of the pycnometer filled with water and filled with both water and wet microspheres, respectively. The quantities m_1, m_2, and m_3 are the mass of the weighing bottle, wet weight of the microspheres, and dried weight of the microspheres, respectively, and ρ_w is the density of water.

Static Adsorption of IgG from Human Plasma

Static adsorption of IgG from human plasma on the gel beads was studied batch-wise. Healthy human blood plasma was obtained from a local plasma donation center and stored at −20 °C in a refrigerator. The plasma samples were thawed and centrifuged at 2000 rpm for 10 min before using. One gram of protein A-immobilizated CL-CM2 and 10 mL of human plasma were incubated at room temperature with gentle shaking for 2 h. After that, the adsorbents were packed in a glass column (144 mm × 10 mm) and irrigated with lactated ringer's solution (0.02 M PBS, pH = 7.4) to wash away the residue plasma. A peristaltic pump was used to adjust the velocity to 4 mL/min, and the column effluent was monitored by UV detection at 280 nm. When the reading was reduced to 0, an appropriate amount of eluent (0.02 M citric acid, pH = 2.5) was used to elute the captured IgG until the absorbance (at 280 nm) decreased to zero. The amount of bound and eluted IgG was

determined using a UV-Visible spectrophotometer (UV-2450, Japan) at 280 nm. The extinction coefficients used were 1.38 mL. $mg^{-1}\cdot cm^{-1}$ for IgG. The eluted acidic fractions were immediately neutralized with a Tris base (0.193 g Tris/50 mL eluent). The purity of IgG in the eluted fractions was checked by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions.

Dynamic Adsorption of IgG from Human Plasma

To evaluate the dynamic adsorption capacity of IgG from human plasma, 2.8 g of protein A-immobilized CL-CM2 suspension in lactated ringer's solution (0.02 M PBS, pH = 7.4) were packed into a glass column (144 mm × 10 mm). Thirty milliliters of lactated ringer's solution was applied to equilibrate the column at a rate of 4 mL/min, which was controlled by peristaltic pump. Subsequently, 20 mL of thawed plasma was perfused into the column at a 2 mL/min flow rate. Lactated ringer's solution was used to wash away the nonspecific adsorption of plasma. After the absorbance (at 280 nm) decreased to 0, the elution buffer (0.02 M citric acid, pH = 2.5) was used to elute the captured IgG until the absorbance decreased to 0. After the operation finished, the column was washed off by lactated ringer's solution and deionized water, successively, and then stored at 4 °C in 0.1% (m/v) sodium azide aqueous solution to prevent bacterial growth. The measurement of IgG was similar to that mentioned in the above section.

RESULTS AND DISCUSSION

Morphology of Cross-linked Cellulose Microspheres

The preparation of CL-CM2 included both physical and chemical crosslinking. The nucleophilic substitution reaction between cellulose and epichlorohydrin takes place under alkaline conditions. Figures 1A and 1B show the SEM image of CL-CM2, in which one can see that CL-CM2 presented an excellent spherical shape and the microspheres exhibited a porous surface structure. A good spherical shape is important for outstanding permeability and hydraulics performance. Figure 1C shows the size distribution of CL-CM2, in which one can see the size of 90% of CL-CM2 distributed between 80 μm and 210 μm. The mean diameter of the microspheres was about 150 μm. Microspheres with a size of 100 to 200 μm were chosen as protein A carrier in this case (Cao *et al.* 2007). The cellulose particle size and distribution could be controlled by changing parameters such as the concentration of the cellulose solution, dispersant dosage, oil-to-water ratio, and stirring speed (Lenfeld *et al*. 1992).

Physical Properties of CL-CMs

Figure 2 shows the effect of epichlorohydrin content on the morphology and dispersion of CL-CMs. When the added volume of epichlorohydrin was less than 3% of the cellulose solution (V/V), microspheres without a good spherical shape were formed. When the volume of epichlorohydrin was greater than 8%, the microspheres appeared to aggregate obviously and without good dispersion. The main reason was that excess epichlorohydrin resulted in crosslinking among the microspheres. Figure 3 shows the influence of epichlorohydrin content on the pore volume of CL-CMs. The average pore volume of CL-CMs decreased from 13.5 to 9.6 mL/g as the epichlorohydrin content increased from 3% to 8%. This can be explained by the fact that the additional

epichlorohydrin led to a higher crosslinking degree of cellulose microspheres, which resulted in smaller pore volumes.

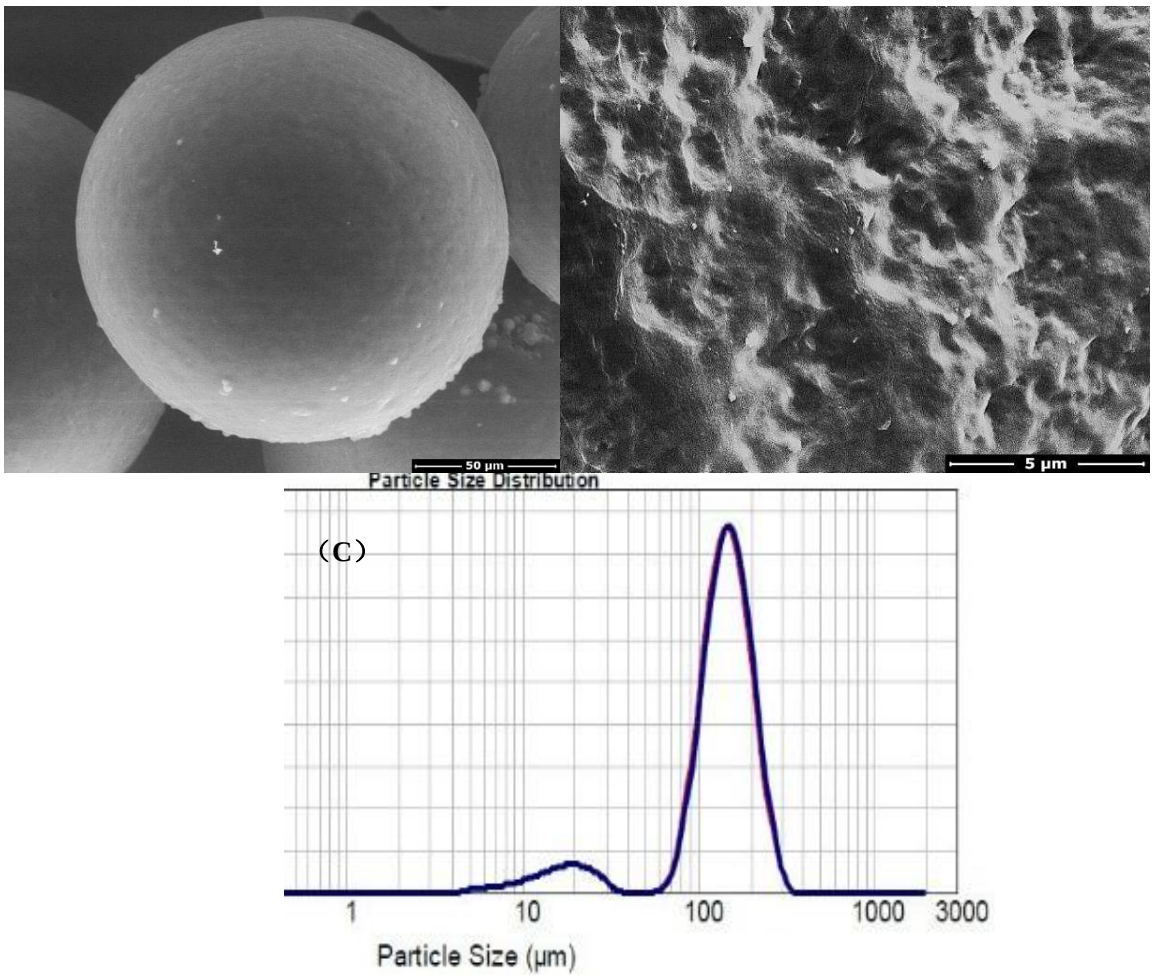

Fig. 1. SEM images and size distribution of CL-CM2: (a) morphology of CL-CM2 (the scale bar: 50 μm); (b) surface structure of CL-CM2 (the scale bar: 5μm); (c) size distribution of CL-CM2

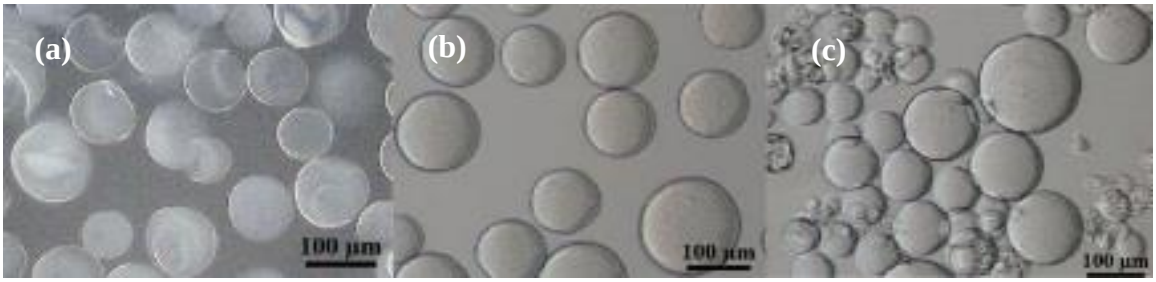

Fig. 2. Effect of epichlorohydrin content on the appearance and dispersity of CL-CMs: (a) 2%; (b) 5%; (c) 9%

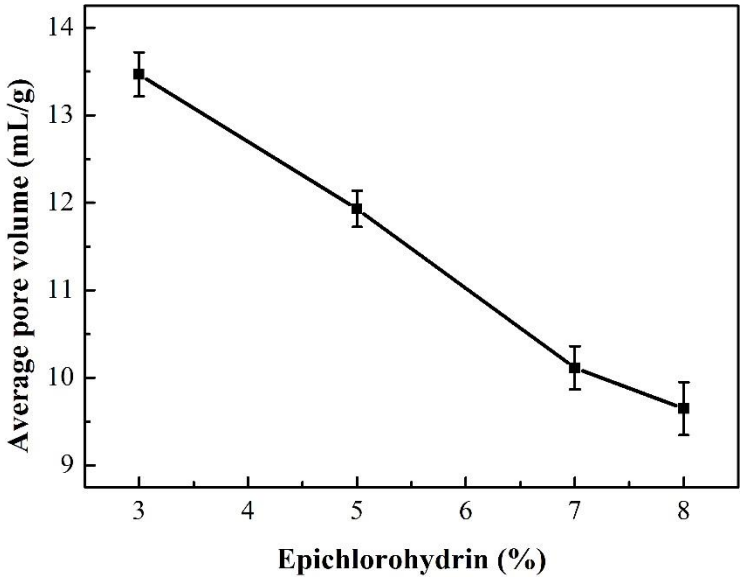

Fig. 3. Effect of epichlorohydrin amount on average pore volume of CL-CMs

Wet real density and moisture content were also measured, and the corresponding results are shown in Table 1. Generally, wet real density represents the quality of unit volume of wet microspheres. The wet real density remained near 1.03 g/mL for all of the microspheres, which indicated that CL-CMs could be used as column packing. The moisture content was more than 90%, suggesting that the cellulose microspheres had high hydrophilicity. As the epichlorohydrin content increased, the wet real density increased, while moisture content decreased. This may have occurred because the crosslinking degree was enhanced with increasing amounts of epichlorohydrin, which resulted in a decrease in water content in the cellulose microspheres.

Table 1. Effects of Epichlorohydrin Content on Wet Real Density and Moisture Content of CL-CMs

Epichlorohydrin (%)	3	5	7	8
Wet real density (g/mL)	1.003	1.015	1.048	1.052
Standard deviations	0.010	0.009	0.018	0.012
Moisture content (%)	93.2	92.3	91.1	90.6
Standard deviations	1.93	2.34	1.53	1.89

Adsorption of IgG from Human Plasma

Protein A immobilization

To couple protein A with the surface of wet CL-CM2, $NaIO_4$ was used to convert the hydroxyl groups of cellulose into aldehyde groups. Figure 4 shows the content of aldehyde groups on microspheres oxidized by various concentrations of $NaIO_4$. The number of aldehyde groups increased from 44 to 161 μmol/g with an increase in sodium periodates concentration from 0.01 to 0.05 M. The microspheres were activated with five different $NaIO_4$ concentrations for further study of the correlation between aldehyde group content and protein A immobilization.

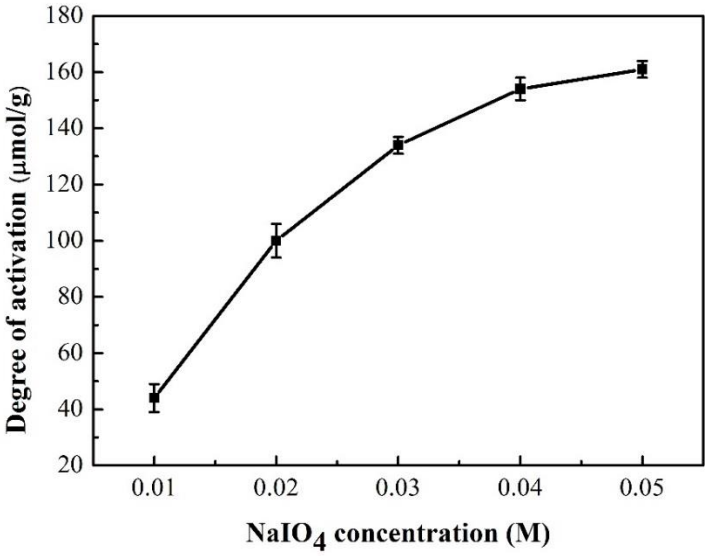

Fig. 4. Effect of $NaIO_4$ concentration on degree of activation

Protein A was immobilized onto the activated cellulose microspheres through the reaction between amino and aldehyde groups, which formed Schiff's base, and then reduced by $NaBH_4$. In this case, the original concentration of protein A added was set to 10 mg/mL. Figure 5 shows the effect of aldehyde group content on protein A immobilization. The amount of protein A immobilization ranged from 1.5 to 9.1 mg per gram of microspheres with an increase in $NaIO_4$ concentration from 0.01 to 0.05 M because of the increasing number of aldehyde groups. When the $NaIO_4$ concentration reached 0.04 M, the coupling efficiency of protein A was higher than 80%. Considering the mechanical strength of cellulose microspheres, 0.04 M $NaIO_4$ was selected for activation in the following experiment, as too much $NaIO_4$ may destroy the skeleton of cellulose because of the ring-opening of the pyran ring.

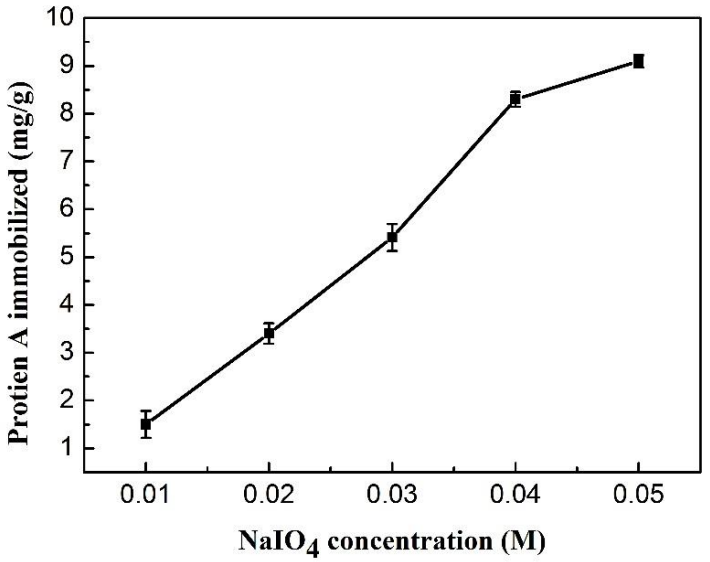

Fig. 5. Effect of $NaIO_4$ concentration on protein A immobilization

The amount of protein A immobilization has an influence on the IgG adsorption behavior. To determine the optimum coupling value, adsorbents containing various amounts of protein A were synthesized after the cellulose microspheres were activated by 0.04 M $NaIO_4$ as mentioned above. Table 2 shows the effect of initial protein A concentration on protein A immobilization and coupling efficiency. Protein A immobilization increased from 1.82 to 9.6 mg/g as the initial protein A concentration increased from 2 to 12 mg/mL, while the efficiency of protein A immobilization decreased slowly. With the increasing of the initial amount of protein A, the interaction probability between protein A and activated groups on cellulose microspheres was raised, leading to the increasing of immobilization amount. However, the coupling efficiency decreased with the increased initial amount of protein A because the quantity of activated groups was fixed. Even in the case of 12 mg/mL, the efficiency of protein A immobilization still had a high coupling rate of higher than 80%.This indicated that cellulose microspheres activated with $NaIO_4$ possess a high protein A coupling efficiency.

Table 2. Effects of Initial Protein A Concentration on Protein A Immobilization and Coupling Efficiency

Protein A concentration (mg/mL)	2	4	6	8	10	12
Protein A immobilization content (mg/g)	1.82	3.56	5.14	6.81	8.32	9.64
Coupling efficiency (%)	94	89	86	85	83	81

Adsorption of IgG with functionalized cross-linked cellulose microspheres

The static adsorption experiments were carried out batch-wise at room temperature by incubating 10 mL of thawed plasma and 1 g of protein A-immobilizated CL-CM2 carrying various amounts of protein A. Figure 6 shows the effect of protein A immobilization concentration on IgG adsorption. The rate of IgG adsorption continued to increase when protein A immobilization content increased from 1.82 to 6.8 mg/g, and then it began to decrease when the protein A immobilization content continued to rise.

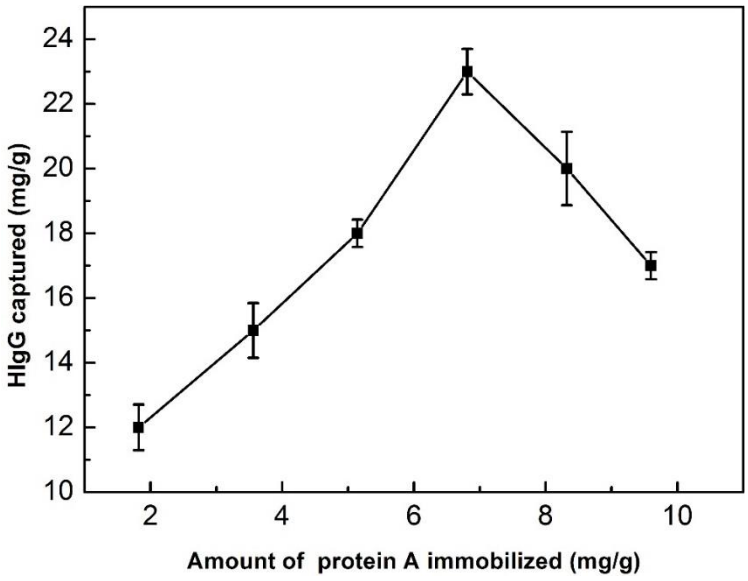

Fig. 6. Effect of protein A coupling content on IgG static adsorption

The maximum adsorption capacity for IgG was 23 mg/g (approximately 19 mg per mL). The reason for this might be that the steric hindrance would hinder the interaction between protein A and HIgG molecules with the increase of protein A immobilization content, which has also been stated by others (Cuatrecass *et al.* 1968; Weiner *et al*. 1994). The protein A-immobilized CL-CM2 showed a high adsorption capacity for IgG and was obviously superior to protein A magnetic cellulose microsphere beads (reported to be 9.12 mg of IgG per mL) (Cao *et al*. 2007).

There is no doubt that adsorption time affects the dynamics of protein binding, including loss of capacity and rate of protein uptake (Cao *et al*. 2007). The time of contact between the plasma and adsorption material is required to be within 2 to 6 h in clinical practice. It is a matter of great importance to determine whether the adsorption equilibrium time of the sorbents meets the clinical requirement or not. Figure 7 shows the effect of adsorption time on IgG static adsorption. Based on the data above, the CL-CM2 containing 6.8 mg of protein A per gram of microspheres was selected for the study of adsorption equilibrium. The maximum adsorption capacity of IgG was obtained after 50 min of incubation, and the adsorption capacity of IgG remained at a constant value with increasing time, which indicated that the adsorption equilibrium was reached. This shows rapid adsorption of IgG by protein A-immobilized CL-CM2.

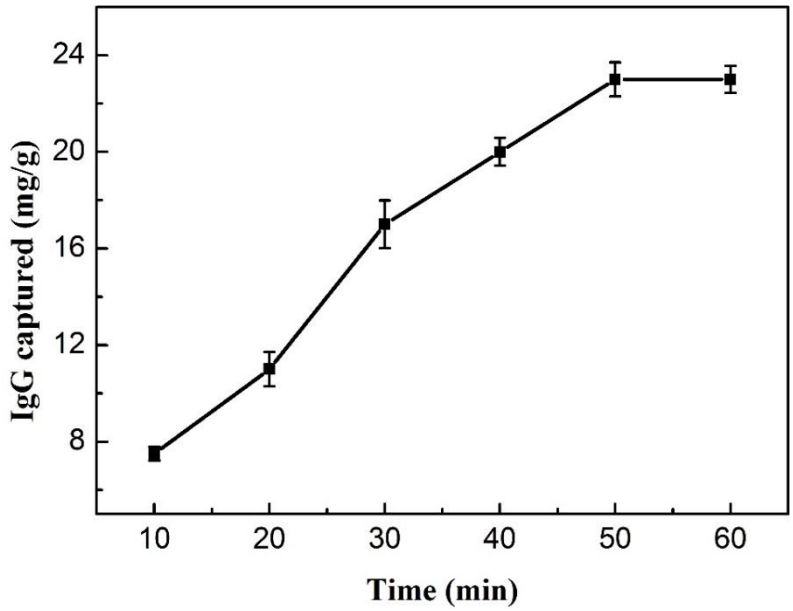

Fig. 7. Effect of static adsorption time on IgG adsorption

To study the dynamic adsorption quantity of IgG with protein A-immobilized CL-CM2, a glass column with 2.8 g of adsorbents was used in the experiment, and the plasma flow rate was 2 mL/min. Figure 8 shows the effect of protein A immobilization on IgG dynamic adsorption capacity.

The amount of IgG captured increased from 5 to 13 mg/g with increasing content of protein A immobilized from 1.82 to 6.8 mg/g, and then the amount of IgG captured decreased when the content of protein A immobilization was further increased from 6.8 to 9.8 mg/g.

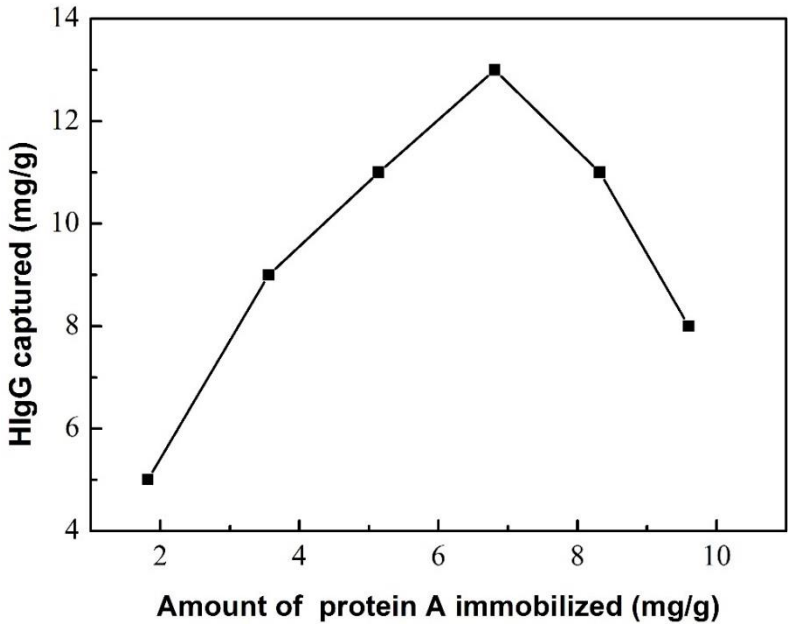

Fig. 8. Effect of protein A coupling content on IgG dynamic adsorption

CL-CM2 without immobilizing protein A acted as a control group compared with the static adsorption and dynamic adsorption of the immunoadsorbent immobilizing 6.8 mg/g of protein A. Figure 9 shows the IgG adsorption properties of the two adsorbents. Without immobilizing protein A onto the surface of microspheres, CL-CM2 only statically adsorbed 1.2 mg/g of IgG and dynamically adsorbed 0.7 mg/g of IgG, indicating that protein A played an important role in IgG adsorption. The adsorption of IgG in dynamic conditions was lower than that in static conditions due to the insufficient contact resulting in the unsaturated adsorption of IgG. The reason was that in dynamic adsorption, the contact time between the plasma and absorbers was not enough because of the flowing of plasma.

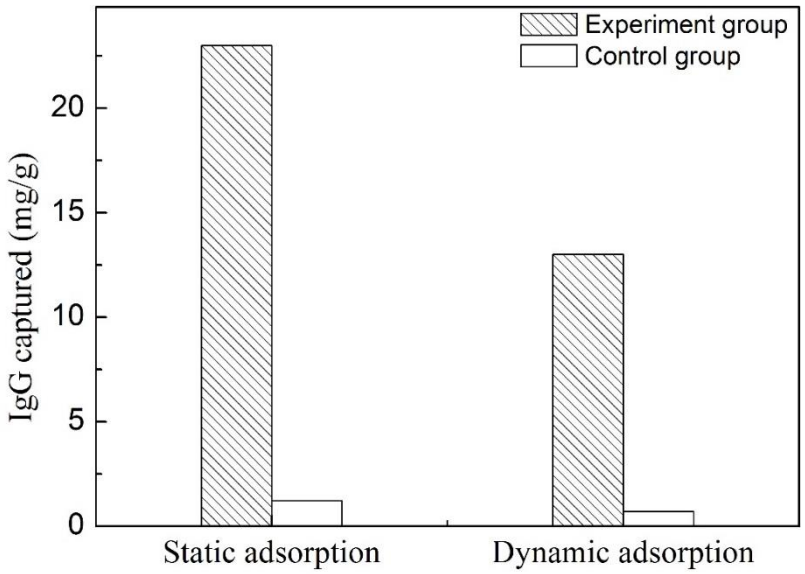

Fig. 9. IgG adsorption of the two adsorbents

Figure 10 shows the SDS-PAGE analysis of eluted materials from adsorbents after static adsorption. The IgG molecule consists of two identical heavy chains and two identical light chains. The molecular weight of the heavy chain is approximately 55 kDa, while the light chain is approximately 25 kDa. The bands of the eluted fraction in Fig. 10 show that the main ingredient of the eluent was IgG, indicating that the adsorbents showed high-selectivity for adsorption of IgG.

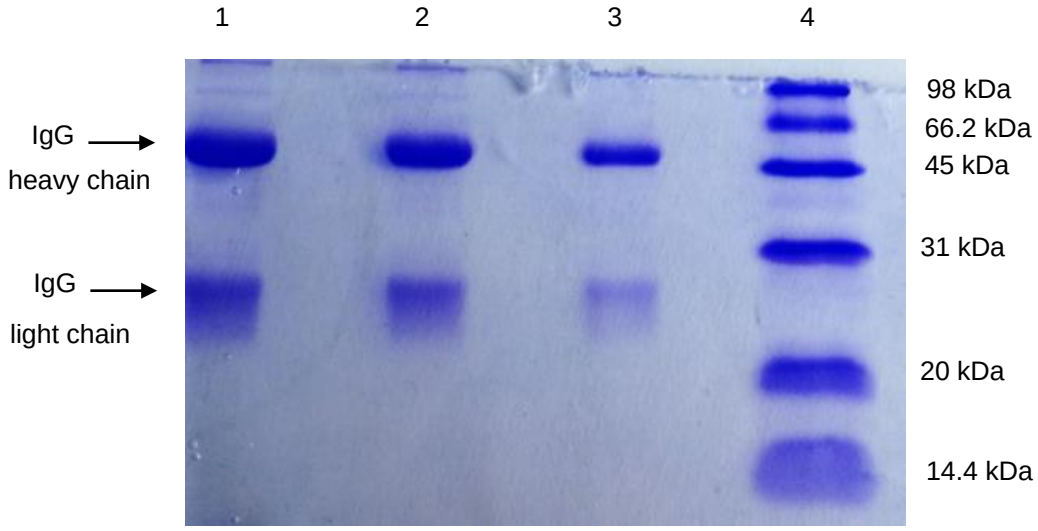

Fig. 10. SDS-PAGE analysis of eluted materials from adsorbents. Lanes 1 to 3 contain materials eluted from adsorbents with different amounts loaded on each lane; lane 4 shows different molecular mass markers with sizes in kDa indicated.

CONCLUSIONS

1. Cross-linked cellulose microspheres (CL-CMs) were successfully prepared using the inverse crosslinking emulsion method from cellulose solution after being dissolved in an alkali hydroxide/urea system, using epichlorohydrin as a crosslinker during the process of emulsification. The cellulose microspheres had a strong spherical shape.
2. Protein A immunoadsorbent was synthesized using $NaIO_4$ to activate CL-CMs to promote the covalent immobilization of protein A. The adsorbents activated by 0.04 M $NaIO_4$ carrying different amounts of protein A were used in the static adsorption experiment and dynamic adsorption experiment, respectively, to remove IgG from human plasma. Maximum IgG adsorptions up to 23 mg/g and 13 mg/g were achieved for static adsorption and dynamic adsorption, respectively.
3. The synthetic protein A-immobilized CL-CMs has potential applications in the field of blood purification.

ACKNOWLEDGMENTS

This work was financially supported by the National Natural Science Foundation of China (Grant Nos. 51372085 and 21464017) and Fundamental Research Funds for the Central Universities.

REFERENCES CITED

Braun, N., and Boseh, T. (2000). "Immunoadsorption current status and future developments," *Expert Opinion on Investigational Drugs* 9(9), 2017-2038. DOI: 10.1517/13543784.9.9.2017

Braun, N et al. (2000). Klein, E. C., Klein, R., Kötter, I., Saal, J., and Risler, T. (2000). "Immunoadsorption onto protein A induces remission in severe systemic lupus erythematosus," *Nephrology Dialysis Transplantation* 15(9), 1367-1372. DOI: 10.1093/ndt/15.9.1367

Cai, J., and Zhang, L. N. (2005). "Rapid dissolution of cellulose in LiOH/urea and NaOH/urea aqueous solutions," *Macromolecular Bioscience* 5(6), 539-548. DOI: 10.1002/mabi.200400222

Cao, Y., Tian, W., Gao, S., Yu, Y., Yang, W., and Bai, G. (2007). "Immobilization staphylococcal protein A on magnetic cellulose microspheres for IgG affinity purification," *Artificial Cells Blood Substitutes Immobilization Biotechnol.* 35(5), 1-14. DOI: 10.1080/10731190601188331

Cuatrecass, P., Wilcek, M., and Anfinsen, C. B. (1968). "Selective enzyme purification by affinity chromatography," *Proceedings of the National Academy of Sciences* 61(2), 636-642. DOI: 10.1073/pnas.61.2.636

Ettenauer, M., Loth, F., Thummler, K., Fischer, K., Weber, V., and Falkenhagen, D. (2011). "Characterization and functionalization of cellulose microbeads for extracorporeal blood purification," *Cellulose* 18(5), 1257-1263. DOI: 10.1007/s10570-011-9567-2

Fukuzumi, H., Saito, T., Wata, T., Kumamoto, Y., and Isogai, A. (2009). "Transparent and high gas barrier films of cellulose nanofibers prepared by TEMPO-mediated oxidation," *Biomacromolecules* 10(1), 162-165. DOI: 10.1021/bm801065u

Gericke, M., Trygg, J., and Fardim, P. (2013). "Functional cellulose beads: Preparation, characterization, and applications," *Chemical Reviews* 113(7), 4812-4836. DOI: 10.1021/cr300242j

Hober, S., Nord, K., and Linhult, M. J. (2007). "Protein A chromatography for antibody purification," *Journal of Chromatography B* 848(1), 40-47. DOI: 10.1016/j.jchromb.2006.09.030

Jaulmes, A., Vidal, M. C., and Pantazaki, A. (2001). "Adsorption kinetics of human serum albumin on various types of chromatographic support," *Chromatographia* 53(2), S417-S423. DOI: 10.1007/BF02490368

Jungbauer, A., and Hahn, R. (2004). "Engineering protein A affinity chromatography," *Current Opinion in Drug Discovery & Development* 7(2), 248-256.

Klemm, D., Heublein, B., Fink, H. P., and Bohn, A. (2005). "Cellulose: Fascinating biopolymer and sustainable raw material," *Angewandte Chemic-International Edition* 44(22), 3358-3393. DOI: 10.1002/anie.200460587

Kong, D. L., Dai, J., and Chen, C. Z. (2000). "Coupling of DNA to bead type cellulose as immunoadsorbent," *Chemical Journal of Chinese Universities* 21(12), 1848-1851.

Kundu, S. K., and Roy, S. K. (1979). "Aminopropyl silica gel as a solid support for preparation of glycolipid immunoadsorbent and purification of antibodies," *Journal of Lipid Research* 20(7), 825-833.

Langone, J. J. (1982). "Protein A of *Staphylococcus aureus* and related immunoglobulin receptors produced by streptococci and pneumococci," *Advances in Immunology* 32, 157-252.

Lenfeld, J., Peska, J., and Stamberg, J. (1992). "Preparation of porous bead cellulose with technical grain size," *Angewandte Makromolekulare Chemie* 197, 201-206.

Luo, X. G., and Zhang, L. N. (2010). "Creation of regenerated cellulose microspheres with diameter ranging from micron to millimeter for chromatography applications," *Journal of Chromatography A* 1217(38), 5922-5929. DOI: 10.1016/j.chroma.2010.07.026

Luo, X. G., and Zhang, L. N. (2013). "New solvents and functional materials prepared from cellulose solutions in alkali/urea aqueous system," *Food Research International* 52(1), 387-400. DOI: 10.1016/j.foodres.2010.05.016

Mao, Y., Zhou, J., Cai, J., and Zhang, L. N. (2006). "Effects of coagulants on porous structure of membranes prepared from cellulose in NaOH/urea aqueous solution," *Journal of Membrane Science* 279(1-2), 246-255. DOI: 10.1016/j.memsci.2005.07.048

Mohanty, A. K., Misra, M., and Drzal, L. T. J. (2002). "Sustainable bio-composites from renewable resources: Opportunities and challenges in the green materials world," *Journal of Polymers and the Environment* 10(1), 19-26. DOI: 10.1023/A:1021013921916

O'Neill, J. J., and Reichardt, E. P. (1951). "Method of producing cellulose pellets," United States Patent No. 2,543,928.

Richman, D. D., Cleveland, P. H., Oxman, M. N., and Johnson K. M. (1982). "The binding of staphylococcal protein A by the sera of different animal species," *Journal of Immunology* 128(5), 2300-2305.

Shtilman, M. I. (1993). *Immobilization on Polymers*, *New Concepts in Polymer Science*, VSP International Science Publishers, Holland.

Somnier, F. E., and Langvad, E. (1989). "Plasma exchange with selective immunoadsorption of anti-acetylcholine receptor antibodies," *Journal of Neuroimmunology* 22(2), 123-127. DOI: 10.1016/0165-5728(89)90042-8

Walker, J. M. (2002). *The Protein Protocols Handbook*, 2nd Edition, Human Press, Totowa, NJ.

Weiner, C., Sara, M., and Sleytr, U. B. (1994). "Novel protein A affinity matrix prepared from 2-dimensional protein crystals," *Biotechnology and Bioengineering* 43(4), 321-330. DOI: 10.1002/bit.260430409

Woodings, C. (2001). *Regenerated Cellulose Fibres*, Woodhead Publishing, Cambridge, UK.

Wu, J. J., Zhao, N., Zhang, X. L., and Xu, J. (2012). "Cellulose/silver nanoparticles composite microspheres: Eco-friendly synthesis and catalytic application," *Cellulose* 19(4), 1239-1249. DOI: 10.1007/s10570-012-9731-3

Article submitted: October 28, 2015; Peer review completed: February 27, 2016; Revised version received and accepted: March 29, 2016; Published: April 14, 2016.
DOI: 10.15376/biores.11.2.4885-4898