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Pathogenic antibody removal using magnetically stabilized fluidized bed

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Abstract

Magnetic poly(2-hydroxyethyl methacrylate) (mPHEMA) beads were used in the removal of anti-dsDNA antibodies from systemic lupus erythematosus (SLE) patient plasma in a magnetically stabilized fluidized bed. mPHEMA beads, in the size range of 80–120 μm, were produced by suspension technique. Then, DNA was immobilized onto mPHEMA beads by carbodiimide activation. Magnetic beads were contacted with blood in in vitro systems. Loss of blood cells and clotting times were followed. mPHEMA beads were characterized by scanning electron microscopy (SEM). Important results obtained in this study are as follows: the mPHEMA beads have a spherical shape and porous structure. Loss of cells in the blood contacting with mPHEMA/DNA was negligible. The anti-dsDNA adsorption capacity decreased significantly with the increase of the flow-rate. With increasing anti-dsDNA antibody concentration, the amount of antibody adsorbed per unit mass increased, then reached saturation. Maximum anti-dsDNA antibody adsorption capacity was found to be 97.8 mg/g. Pathogenic antibody molecules could be repeatedly adsorbed and desorbed with these magnetic beads without noticeable loss in their antibody adsorption capacity. Because of the good blood-compatibility, mPHEMA is hopeful for the treatment of SLE by magnetically stabilized fluidized bed systems in the future.

Keywords: Antibody removal; Magnetic adsorbents; Affinity beads; DNA; SLE

1. Introduction

Anti-double-stranded DNA antibodies were first described in the sera of patients with systemic lupus erythematosus (SLE) more than 20 years ago [1]. Since then, dsDNA antibodies have emerged as a central focus in the investigation of the pathogenesis of SLE and of autoimmunity in general. Antibodies against DNA serve as markers of diagnostic and prognostic significance in SLE, and there is compelling evidence for an association between anti-dsDNA antibodies and tissue damage [2]. Since many of the clinical manifestations of this disease can be attributed to immune complex deposition, the concept has arisen that antibodies against DNA mediate tissue damage by the formation of DNA-anti-dsDNA antibody immune complexes which localize throughout the body, most prominently in the kidneys [3]. However, this model, while consistent with many clinical and serologic findings, has been difficult to verify. For example, although there is suggestive evidence for DNA-anti-dsDNA antibody complexes in patient sera, such complexes have not been demonstrated either consistently or conclusively [4].

The level of anti-dsDNA antibodies correlates well with the disease activity and organ involvements, such as nephritis and cerabritis. In such cases the removal of anti-dsDNA antibodies from plasma may lead to a clinical improvement. Analysis of plasmapheresis has shown that plasma exchange acts by removing autoantibodies [5]. But, risks of plasmapheresis therapy stem from the nonspecific elimination of all plasma components according to their plasma content. Necessary substitutions may induce allergic reactions and plasma replacement can convey infective diseases. Because of the disadvantages of plasmapheresis, efforts were made to develop a more specific extracorporeal technique to remove the pathogenic substances from plasma. Extracorporeal immunoadsorption with affinity adsorbents has become increasingly utilized a therapeutic modality to remove pathogenic antibodies containing from plasma of patients. The first ex-vivo application

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of DNA-attached adsorbents was achieved by Terman et al. [6]. They removed anti-dsDNA antibodies from the plasma of positively immunized rabbits by circulating their blood through an extracorporeal shunt containing immobilized DNA. The first clinical trial of immunoadsorption was also done by Terman et al., in which they used DNA-affinity adsorbents to treat a female patient suffering from SLE [7]. Nicolaev et al. have applied DNA-immobilized activated carbon hemoperfusion column to the treatment of patients with psoriasis [8]. Ventura et al. have utilized poly(ethylene vinyl alcohol) hollow fiber carrying histidine for the removal of anti-dsDNA antibodies in in vitro system [9]. Zhu et al. [10] have used DNA-immobilized non-woven poly(ethylene terephthalate) fabric fibers for treatment of systemic lupus eryhtematosus [10]. Recently, Yu and He have prepared DNA-immobilized hydroxy-ethyl cellulose crosslinked chitosan beads as immunoadsorbents for specific removal of anti-dsDNA antibodies in SLE serum [11]. Kato and Ikada have reported anti-dsDNA antibody adsorption using DNA-carrying poly(ethylene terephthalate) microfibers [12].

There have been several separation approaches performed under magnetic field [13–15]. The most well known technique is the magnetically stabilized fluidized bed. Magnetically stabilized fluidized bed exhibits combination of the best characteristics of both packed and fluidized bed. These include the efficient fluid-solid mass transfer properties, elimination of particle mixing, low pressure drop, high feed-stream solid tolerances, good fluid-solid contact, elimination of clogging and continuous countercurrent operation [16]. Especially, when dealing with highly viscous mediums, such as blood contact with the magnetic adsorbent in a magnetically stabilized fluidized bed is desirable because of high convective transport rates without cell damage (i.e., hemolysis). Recently, there has been increased interest in the use of magnetic adsorbents in biomolecule coupling and nucleic acid purification [17]. Magnetic adsorbents can be produced using inorganic materials or polymers. High mechanical resistance, insolubility and excellent shelf life make inorganic materials ideal as adsorbent. The main disadvantage of inorganic supports is their limited functional groups for ligand coupling. Magnetic adsorbents can be porous or non-porous. They are more commonly manufactured from polymers since they have a variety of surface functional groups which can be tailored to use in different biomedical applications [18–22].

In this study, we have assessed the suitability of a magnetically stabilized fluidized bed utilising mPHEMA/DNA beads for the removal of anti-dsDNA antibodies from SLE patient human plasma. We selected magnetic poly(2-hydroxyethyl methacrylate) (mPHEMA) beads as the basic solid-phase, which is one of the most widely used hydrophilic polymers in biomedical applications, by considering possible applications of these sorbents in direct hemoperfusion extracorporeal therapy, in which blood compatibility is one of the main concerns [23–25]. mPHEMA beads were produced by suspension polymerization. Hydroxyl functional groups on the magnetic beads were activated by carbodiimide, and then, DNA molecules were covalently immobilized to the magnetic beads through the active sites. Anti-dsDNA antibody adsorption onto the DNA-affinity beads from the blood plasma of a patient with SLE is discussed.

2. Experimental

2.1. Preparation of magnetic PHEMA beads

The mPHEMA beads were prepared by suspension polymerization in an aqueous medium as described in our previous article [26,27]. Hydroxyethyl methacrylate (HEMA) was purchased from Sigma (St. Louis, MO, USA) and was purified by vacuum distillation under a nitrogen atmosphere. Ethylene glycol dimethacrylate (EGDMA, Merck, Darmstadt, Germany) was used as the crosslinking agent. Magnetite particles (Fe_3O_4, diameter $< 1\,\mu m$) was obtained from Aldrich (USA). All other chemicals were obtained from Merck as analytical grade. A typical suspension copolymerization procedure of mPHEMA beads was given as below: the dispersion medium was prepared by dissolving 200 mg of poly(vinyl alcohol) (PVA; molecular weight: 50,000) within 50 ml of distilled water. The desired amount of 2,2′-azobisiso-butyronitrile (AIBN) (0.06 g) was dissolved within the monomer phase 12.0/4.0/8.0 ml (EGDMA/HEMA/toluene) with 1.0 g magnetite particles. This solution was then transferred into the dispersion medium placed in a magnetically stirred (at a constant stirring rate of 600 rpm) glass polymerization reactor (100 ml) which was in a thermostatic water bath. The reactor was flushed by bubbling nitrogen and then was sealed. The reactor temperature was kept at 65 °C for 4 h. Then the polymerization was completed at 90 °C in 2 h. After polymerization, the mPHEMA beads were separated from the polymerization medium. The residuals (e.g., unconverted monomer, initiator and other ingredients) were removed by a cleaning procedure. Briefly, magnetic beads were transferred into a reservoir, and washing solutions (i.e., a dilute HCI solution, and a water–ethanol mixture) were recirculated through the system which includes also an activated carbon column, to be assured that the magnetic beads were clean. Purity of the magnetic beads was followed by observing the change of optical densities of the samples (λ: 280 nm) taken from the liquid phase in the recirculation system, and also from the DSC thermograms of the magnetic beads obtained by using a differential scanning micro-calorimeter (Mettler, Switzerland). Optical density of uncleaned magnetic beads was 2.63, but after the cleaning operation this value was reduced to zero. In addition, when the thermogram of uncleaned beads was recorded, it had a peak around 60 °C. This peak might originate from AIBN, but after application of the cleaning procedure, no peak between 30 and 100 °C was observed on the thermogram.

2.2. Characterization of mPHEMA beads

2.2.1. Density measurement

The dry density of the magnetic beads were measured with pycnometer by dispersing the dry beads in ethanol.

2.2.2. SEM studies

The surface morphology and internal structure of the mPHEMA beads were observed in a scanning electron micro-

scope (JEOL, JEM 1200EX, Tokyo, Japan). mPHEMA beads were dried at room temperature and coated with a thin layer of gold (about 100 Å) in vacuum and photographed in the electron microscope with ×1000 magnification. The particle size was determined by measuring at least 100 beads on photographs taken on a SEM.

2.2.3. Coagulation time (CT)

Magnetic beads were incubated in 0.1 M phosphate buffer solution (pH 7.4) for 24 h at room temperature and washed on a glass filter with 0.5 M NaCl solution and distilled water. Fresh frozen human plasma (0.1 ml) was preheated to 37 °C for 2 min and then the beads were added into this medium and mixed immediately. The clotting time was measured using the fibrometer method [28].

2.2.4. Activated partial thromboplastin time (APTT)

Magnetic beads were incubated in 0.1 M phosphate buffer solution (pH 7.4) for 24 h at room temperature and washed on a glass filter with 0.5 M NaCl solution and distilled water. Fresh frozen human plasma (0.1 ml) was preheated to 37 °C for 2 min. Partial thromboplastin (0.3 ml, bioMerieux, Marcy-1'Etoile, France) was also preheated to 37 °C for 2 min and was added to preheated human plasma. Then, the beads were transferred into this medium. Thirty seconds later, $CaCl_2$ (0.1 ml, 0.025 M) was added, then, the activated partial thromboplastin time (APTT) was determined using the fibrometer method [28].

2.2.5. Prothrombin time (PT)

In order to determine prothrombin time (PT), a one-stage prothrombin method was used. Magnetic beads were incubated in 0.1 phosphate buffer solution (pH 7.4) for 24 h at room temperature. Fresh frozen human plasma (0.1 ml) was preheated to 37 °C for 2 min. The partial thromboplastin (0.3 ml, bioMerieux, Marcy-1'Etoile, France) was also preheated to 37 °C for 2 min and was added to preheated human plasma. Then, the beads were added into this medium. Thirty seconds later, $CaCl_2$ (0.1 ml, 0.025 M) was transferred into the medium. After these operations, the prothrombin time was measured using the fibrometer method [28].

2.2.6. Cell adhesion studies

Heparinized human blood was incubated with the beads for 1 h. It should be noted that prior to the blood contact, the beads were washed with 0.1 M KCl solution in buffer until no further impurities (monitored by the absorbance at 280 nm) was detected in the washing solution. Blood samples were withdrawn from the beginning and the end of the operation, and the platelet and leukocyte count of samples were determined using a microscope.

2.3. Carbodiimide activation

Prior to the activation process, the mPHEMA beads were kept in distilled water for about 24 h and washed on a glass filter with 0.5 M NaCl solutions and water in order to remove impurities. Carbodiimide aqueous solution (100 ml) (15.0 mg/ml) were prepared in carbonate buffer. The pH of solution was quickly adjusted to 10.5 with 1.0 M NaOH while it was magnetically stirred. One gram of mPHEMA beads was then added to solution and the activation procedure was continued for 24 h. The main problem in surface modification is to prevent deformation and/or destruction of beads. If there is any deformation, encapsulated magnetites (i.e., iron particles) can easily release from the polymeric structure. This decreases the magnetic properties of the polymeric adsorbent. In order to prevent structural destructions/deformations, coupling reactions were carried out under mild experimental conditions and a long reaction period. That is why carbodiimide was used for the incorporation of activated groups on/in to the mPHEMA structures under mild conditions. After the activation reaction, in order to remove the excess activation agent (i.e., carbodiimide), the mPHEMA beads were washed with 0.1 M $NaHCO_3$ and then with distilled water.

2.4. DNA coupling

DNA (from herring testes) was purchased from Sigma. The freshly carbodiimide-activated mPHEMA beads were magnetically stirred at a constant temperature of 20 °C for about 4 h with 10 ml DNA solution. pH and initial carbodiimide concentration on the covalent coupling of DNA were 7.0 and 15 mg/ml, respectively. The initial concentration of DNA in the medium was 1.0 mg/ml. After coupling, the DNA-affinity beads were washed with 0.1 M CH_3COONa (pH 4.5) + 0.15 M NaCl, and finally with 0.1 M NaHCO3 (pH 9.5) + 0.5 M NaCl. The amount of DNA-immobilized on the carbodiimide-activated mPHEMA beads was determined by measuring the decrease of the DNA concentration and also by considering the DNA molecules adsorbed non-specifically (not through the carbodiimide activated sites), by spectrophotometry at 260 nm. Prepared mPHEMA beads were held at 4 °C prior to use.

In order to estimate the amount of released DNA, the affinity beads (250 mg) were placed in test tube containing 10 ml of phosphate buffer solution (pH 7.4) and shaken on a rotary shaker for 24 h. The amount of DNA released into the medium was measured cumulatively as the absorption band at 260 nm by a bench-top spectrophotometer.

2.5. Iron leach studies

In order to estimate the amount of leached iron from the magnetic beads, the beads (250 mg) were placed in test tubes containing 10 ml of leach media and shaken on a rotary shaker for 24 h. The amount of iron leached into the medium was determined by a graphite furnace atomic absorption spectrophotometer (AAS 5EA, Carl Zeiss Technology, Zeiss Analytical Systems, Germany). Three kinds of release media were used: pH 2.0 buffer of acetic acid solution (50%, v/v), phosphate buffer solution (pH 7.0) and sodium citrate/NaOH buffer solution (pH 12.0).

2.6. Anti-dsDNA antibody adsorption from human plasma in MSFB

The breakthrough behaviour of pathogenic antibody (i.e., anti-dsDNA antibody) in the magnetically stabilized fluidized bed of the mPHEMA/DNA beads was investigated by frontal adsorption experiments. Blood samples taken from a patient with SLE was used in these studies. Blood was centrifuged at 500 × *g* for 30 min at room temperature to separate the plasma. The beads suspended in pure water were degassed under reduced pressure (by using water suction pump) and magnetically stabilized into a column (10 cm × 0.9 cm i.d.) equipped with a water jacket for temperature control. The vertically oriented magnetic field was produced by passing DC current through two modified Helmholtz coils (1.5 cm diameter × 2.5 cm thick) spaced 4 cm apart. At a current of 1.6 A (50 W), each coil produced a magnetic field of 40 Gauss. Equilibration of the column was performed by passing four column volumes of phosphate buffer (pH 7.4) before injection of the patient plasma. In a typical adsorption system, 50 ml of the SLE patient plasma was passed through the column containing beads, by a peristaltic pump for 2 h. Dynamic binding capacity (DBC) was calculated from anti-dsDNA antibody breakthrough curves [29]. The amount of anti-dsDNA antibody adsorbed on the mPHEMA/DNA beads was determined by radioimmunoassay (RIA) by measuring the decrease in the anti-dsDNA antibody concentration in the plasma of the patient.

In the first group of experiments, the flow rate of the patient plasma (i.e., 50 ml) was changed between 0.5 and 3.5 ml/min. In the second group of experiments, anti-dsDNA antibody adsorption isotherm was obtained in the magnetically stabilized fluidized bed. Patient plasma containing different amount of anti-dsDNA antibody were used in these experiments. The changes in the anti-dsDNA antibody concentration with time was followed to obtain the adsorption curves. The amount of adsorbed anti-dsDNA antibody per dry magnetic beads was calculated by using the concentrations of the anti-dsDNA antibody in the initial and in the equilibrium. Each experiment was performed in triplicate for quality control and statistical purposes. For each set of data present, standard statistical methods were used to determine the mean values and standard deviations. Confidence intervals of 95% were calculated for each set of samples in order to determine the margin of error.

2.7. Anti-DNA antibody adsorption from SLE plasma in batch system

Blood samples taken from a patient with SLE was used in these studies. Blood was centrifuged at 500 × *g* for 30 min at room temperature to separate the plasma. Hundred milligrams of mPHEMA/DNA beads were incubated with 5 ml of the plasma obtained from the SLE patient at 25 °C for 60 min. The amount of anti-DNA antibody adsorbed on the mPHEMA/DNA beads was determined by radioimmunoassay by measuring the decrease in the anti-DNA antibody concentration in the plasma of the patient. The amount of anti-DNA antibody adsorption per unit mass of the magnetic beads was evaluated by using the mass balance.

2.8. Desorption and repeated use

In all cases, bound anti-dsDNA antibody molecules were desorbed using 1 M NaCl at pH 4.0 in acetate buffer. In a typical desorption experiment, 50 ml of the desorption agent was recirculated through the magnetically stabilized fluidized bed containing affinity beads for 1 h. Anti-dsDNA antibody concentration in the desorption medium was determined by RIA. The desorption ratio was calculated from the amount of anti-dsDNA antibody adsorbed on the beads and the final anti-dsDNA antibody concentration in the desorption medium. In order to test the reusability of the beads, anti-dsDNA antibody adsorption-desorption procedure was repeated ten times by using the same affinity column. When desorption was achieved, the affinity column was finally cleaned up with 50 mM NaOH solution in order to remove the remaining proteins and to regenerate them, and then re-equilibrated with the starting buffer.

3. Results and discussion

3.1. Characteristics of mPHEMA beads

DNA-immobilized mPHEMA beads (80–120 μm in diameter) were prepared as a specific bioaffinity adsorbent for removal of anti-dsDNA antibodies from SLE patient plasma in magnetically stabilized fluidized bed. mPHEMA beads used in this study are rather hydrophilic and cross-linked structures, i.e., hydrogels [30]. The surface morphology and internal structure of non-magnetic PHEMA and mPHEMA beads are exemplified by the scanning electron pictures in Fig. 1. As seen in Fig. 1A, mPHEMA beads have a spherical form and a rough surface containing macropores due to the abrasion of magnetite crystals (diameter < 0.1 μm) during the polymerization procedure. However, the surface of the non-magnetic spherical PHEMA beads contained no macropores (Fig. 1B). The pictures in Fig. 1C and D were taken with broken beads to observe the internal parts of both non-magnetic and magnetic PHEMA beads. The presence of macropores within the bead interior was clearly seen in these photographs. It can be concluded that the mPHEMA beads have a macroporous interior surrounded by a reasonably rough surface, in the dry state. The roughness of the bead surface should be considered as a factor providing an increase in the specific surface area. The macropores reduce diffusional resistance and facilitate mass transfer because of high inner surface area. This also provides higher DNA attachment and enhances the pathogenic antibody removal capacity. On the other hand, non-magnetic PHEMA beads were in the uniform and spherical shape with smooth surface characteristics. The density of mPHEMA beads is 1.45 mg/ml.

Note that the leach of iron was measured in three different kinds of media including acetic acid solution (50%, v/v; pH 2.0), phosphate buffer solution (pH 7.0) and sodium citrate/NaOH buffer solution (pH 12.0). It should be noted that there was no measurable release.

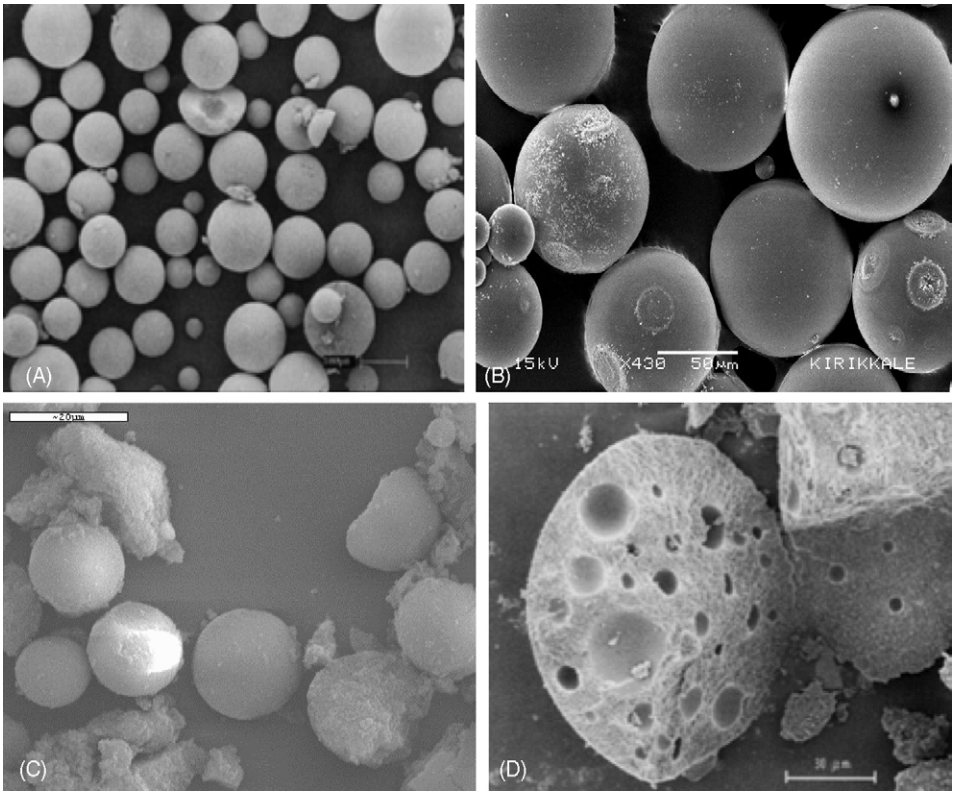

Fig. 1. SEM pictures of polymeric beads: (A) surface of mPHEMA; (B) surface of non-magnetic PHEMA; (C) cross-section of non-magnetic PHEMA; (D) cross-section of mPHEMA.

DNA leakage from the beads was also monitored continuously. There were no DNA leakage in any of the adsorption and desorption media, which assured that the cleaning procedure used for removal of physically adsorbed DNA molecules from the mPHEMA beads was satisfactory.

3.2. Blood compatibility studies

In order to evaluate the blood-compatibility of mPHEMA beads, in vitro coagulation times (CT), prothrombin time and activated partial thromboplastin time (PTT) tests were carried out with mPHEMA beads. The protrombin time (PT) measures the clotting time from the activation of factor VII, through the formation of fibrin clot. This test measures the integrity of the extrinsic and common pathways of coagulation, whereas the activated partial thromboplastin time measures the integrity of the intrinsic and common pathways of coagulation. CT test shows in vitro coagulation time. Table 1 shows the coagulation data. All the clotting times for mPHEMA beads decreased when compared with control plasma. However, these decreases are tolerable by the body. Therefore, we concluded that the blood-compatibility of magnetic beads was rather good, and the clotting times were quite reproducible comparing with the values reported in the related literature [31].

Table 2 summarizes hematological data obtained from in vitro blood assay. Loss of platelet with mPHEMA were 2.6% and 3.5%, respectively. Lost of leukocyte with mPHEMA/DNA were 10.5% and 11.6%, respectively. As seen here, there is no significant cell adhesion on the beads. These observations showed that surfaces of the magnetic beads are resistant to adhesion of -platelets and leukocytes. In conclusion, because of the good non-thrombogenic properties, porous mPHEMA beads seem to be very promising affinity adsorbents for biomedical applications, such as extracorporeal immunoadsorption therapy.

3.3. Anti-dsDNA antibody adsorption

For the extracorporeal removal of pathogenic antibodies by various columns, a wide range of adsorption rates have been

Table 1
Coagulation times of human plasma (reported in sec)[a]

Experiments	APTT	PT	CT
Control plasma	53.6 ± 2.1	22.5 ± 0.7	245 ± 8.6
PHEMA	45.8 ± 1.5	18.6 ± 0.3	226 ± 10.6
PHEMA/DNA	46.9 ± 1.7	20.2 ± 0.5	232 ± 9.5

[a] Each result is the average of six parallel studies.

Table 2
Platelet and leukocyte adhesion with magnetic beads[a]

Substance	Platelet ($\times 10^{-3}$/mm^3)		Leukocyte ($\times 10^{-3}$/mm^3)	
	Initial/final	Loss (%)	Initial/final	Loss (%)
PHEMA	390/380	2.6	4.97/4.45	10.5
PHEMA/DNA	390/376	3.5	4.97/4.39	11.6

[a] Each result is the average of three parallel studies.

reported in the literature. For example, Müller et al. [32] have studied immunoglobulin adsorption in patients with idiopathic dilated cardiomyopathy on a column contained polyclonal anti human immunoglobulin antibodies produced in sheep and each session lasted a mean of 5.6 ± 2.1 h [32]. Jansen et al. have considered 4–5 h as an effective treatment time in their coagulation inhibitors removal studies from patients with haemophilia A, in which they used Ig-Therasorb column contained sepharose coupled with polyclonal sheep antibodies [33]. Schneider et al. studied immunoadsorption plasma perfusion in SLE patients using phenylalanine and tryptophan carrying commercial IM-P columns and reported 2 h treatment time [34]. Some patients with acquired hemophilia have been treated using commercial Immunosorba® column around 5 h effective treatment time [35] for the removal of IgG and inhibitors. Yu and He investigated the adsorption of anti-dsDNA antibodies in SLE serum on DNA immobilized hydroxyethyl crosslinked chitosan beads and reported that equilibrium adsorption time is 2 h [36]. Zhu et al. [37] considered 2 h as a treatment time in their anti-dsDNA antibody adsorption kinetic studies in SLE patient, in which they used poly(ethylene terephthalate) microfibers as adsorbent [37]. Immunoadsorption therapy takes between 2 and 5 h with commercial columns including Prosorba® and Excorim® [38,39]. The flow rate in the aqueous phase, structural properties of adsorbent (e.g., porosity, surface area), amount of adsorbent, adsorbate properties (e.g., molecular dimensions and solubility), initial concentration of antibody determine the adsorption rate. In this study, the SLE patient plasma was passed through the column containing beads for 2 h. The removal rates obtained with the mPHEMA/DNA beads prepared by us seem to be very promising.

3.3.1. Effect of flow-rate

The breakthrough curves at different flow-rates are given in Fig. 2A. Results show that the antibody adsorption amount onto the magnetic beads decreases when the flow rate through the column increases. The adsorption capacity decreased significantly from 97.8 mg/g to 32 mg/g polymer with the increase of the flow-rate from 0.5 ml/min to 3.5 ml/min (Fig. 2B). An increase in the flow rate reduces the plasma volume treated efficiently until breakthrough point and therefore decreases the service time of magnetically stabilized fluidized bed. This is due to decrease in contact time between the anti-dsDNA antibodies and the mPHEMA/DNA beads at higher flow rates. As the adsorption rate is controlled by intra-particle diffusion, an early breakthrough occurs leading to a low bed adsorption capacity. These results are also in agreement with those referred to the literature [40,41]. When the flow rate decreases the contact time in the column is longer, intra particle diffusion then becomes effective. Thus, anti-dsDNA antibodies have more time to diffuse the porous magnetic beads and a better adsorption capacity is obtained.

3.3.2. Effect of anti-dsDNA antibody concentration

Fig. 3 shows the effect of anti-dsDNA antibody concentration on adsorption. As presented in this figure, with increasing anti-dsDNA antibody concentration, the amount of anti-dsDNA

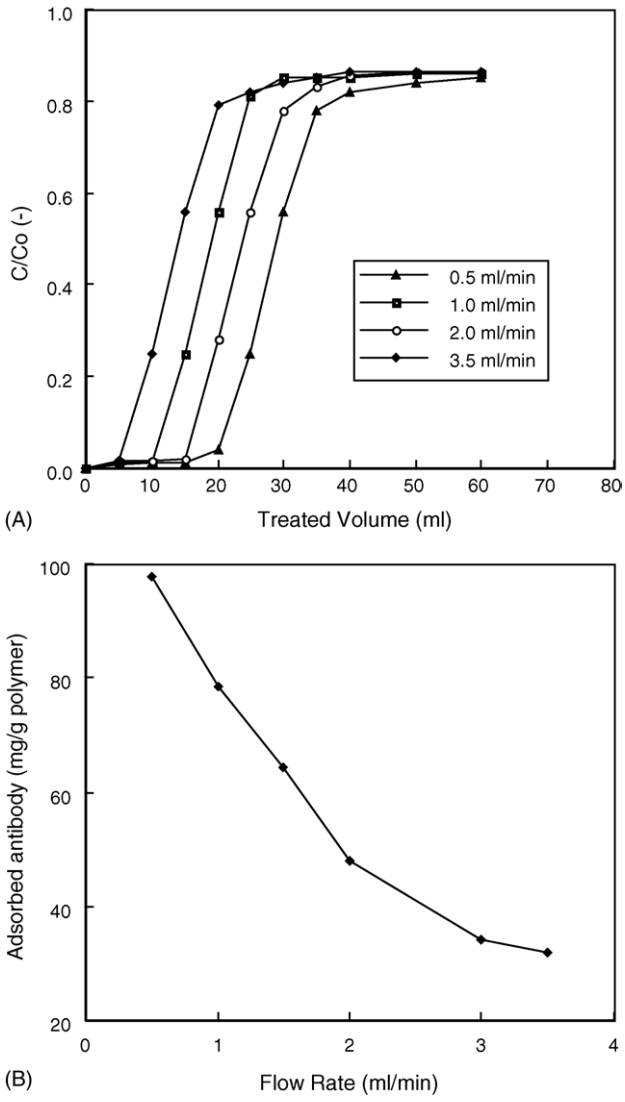

Fig. 2. Effect of flow-rate onto the breakthrough curves (A) and adsorption capacity (B) of anti-dsDNA antibody adsorption: DNA loading: 4.4 mg/g; anti-dsDNA antibody concentration: 1.86 mg/ml; *T*: 25 °C.

antibody adsorbed per unit mass increases below about 1.0 mg/ml, then increases less rapidly. It reached saturation when the protein concentration is greater than 1.5 mg/ml. The steep slope of the initial part of the adsorption isotherm represents a high affinity between anti-dsDNA antibody and incorporated DNA groups. Negligible amount of anti-dsDNA antibody molecules adsorbed on the hydrophilic mPHEMA beads, which was about 0.14 mg/g. DNA incorporation significantly increased the anti-dsDNA antibody adsorption capacity of the magnetic beads up to 97.8 mg/g. It is clear that this increase is due to specific interaction between DNA and anti-dsDNA antibody molecules.

3.3.3. Comparison of magnetically stabilized fluidized bed and batch system

Column-type continuous flow operations appear to have a distinct advantage over batch type operations because the rate of adsorption depends on the concentration of solute in solution

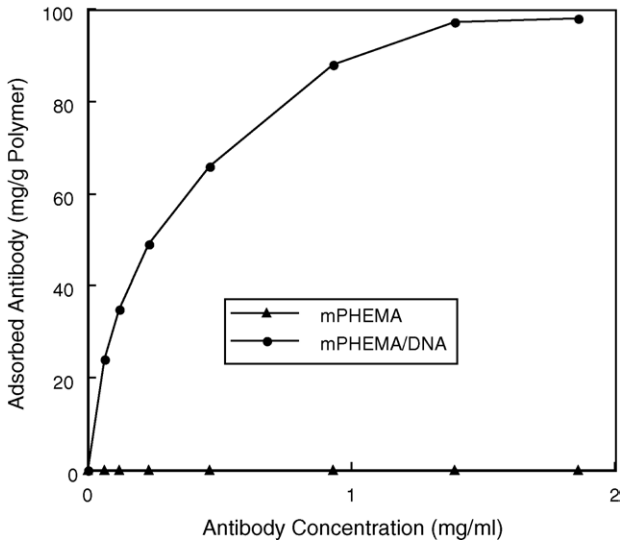

Fig. 3. Effect of anti-dsDNA antibody concentration on adsorption capacity; DNA loading: 4.4 m/g; flow-rate: 0.5 ml/min; *T*: 25 °C.

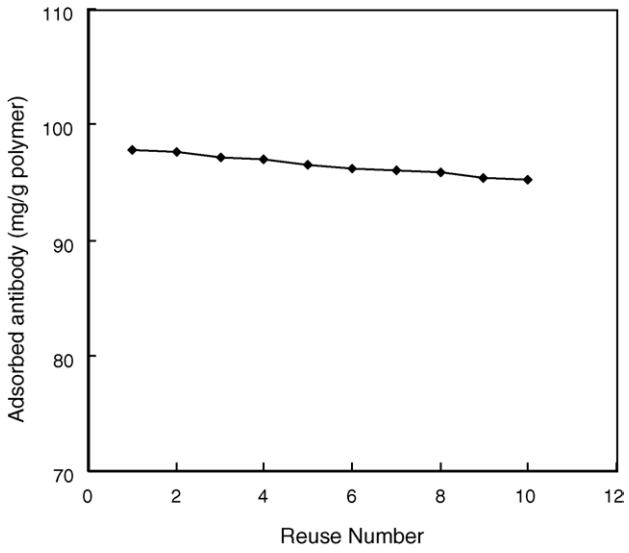

Fig. 4. Reusability of mPHEMA/DNA beads. DNA loading: 4.4 mg/g; flow-rate: 0.5 ml/min; anti-dsDNA antibody concentration: 1.86 mg/ml; *T*: 25 °C.

being treated [42]. For column operation the adsorbents are continuously in contact with a fresh solution. Consequently, the concentration in the solution in contact with a given layer of adsorbent in a column is relatively constant. For batch treatment, the concentration of solute in contact with a specific quantity of adsorbent steadily decreases as adsorption proceeds, thereby decreasing the effectiveness of the adsorbent for removing the solute. Table 3 compares the adsorption capacity of anti-dsDNA antibody in batch and MSFB column operations. The MSFB column capacity was found to be higher than the batch capacity as shown in Table 3. This means, in equilibrium binding experiments, maximum adsorption capacity was 14% lower as compared to the value obtained in MSFB. The higher column capacity may be due to the fact that the continuously large concentration gradient at the interface zones occurred as to passes through the column, while the concentration gradient decreases with time in batch experiments.

3.3.4. Desorption and regeneration of beads

Desorption of anti-dsDNA antibody from mPHEMA/DNA beads was also carried out in magnetically stabilized fluidized bed using 1 M NaCl at pH 4.0 in acetate buffer. The magnetic beads adsorbed with the different amounts of anti-dsDNA antibodies were placed within the magnetically stabilized column, and the amount of anti-dsDNA antibody desorbed in 1 h was determined. The higher desorption ratio was obtained using 1.0 M NaCl at pH 4.0 in acetate buffer after one adsorption-desorption cycle. These results may be contributed to desorption medium containing 1.0 M NaCl at pH 4.0 in acetate buffer might change the charge of the peptide side groups, due to their isoelectric points, resulting in the release of the anti-dsDNA antibody molecules from the matrix. Desorption of anti-dsDNA antibody is achieved under relatively mild conditions (pH 4.0) compared to conditions employed during protein A affinity adsorbents.

Table 3
Adsorption capacity of anti-dsDNA antibodies in batch and MSFB column operations

Batch capacity (mg/g)	Column capacity (mg/g)
85.8 ± 1.4	97.8 ± 1.6

In order to show the reusability of the mPHEMA/DNA beads, the adsorption-desorption cycle was repeated ten times using the column. For sterilization, after each adsorption-desorption cycle, column was washed with 50 mM NaOH solution for 30 min. After this procedure, column was washed with distilled water for 30 min, then equilibrated with the phosphate buffer for the next adsorption-desorption cycle. At the end of ten adsorption-desorption cycles, anti-dsDNA antibody adsorption capacity decreased 2.6% using 1.0 M NaCl at pH 4.0 in acetate buffer (Fig. 4).

4. Conclusions

Conventional immunoadsorption methods based on the use of a combination of classical chromatographic techniques are very time consuming [43–45]. Magnetically stabilized fluidized bed enables the use of magnetic processing for rapid and selective removal. Magnetic separation has several potential advantages over conventional approaches. Magnetically stabilized fluidized bed cartridges require high flow-rates with a much lower operating pressure than a packed bed column. In this technique, the biomolecule to be separated can be directly transported by convection to the ligand immobilized on the surface of the beads, higher throughput and faster processing times onto the affinity beads can be achieved. mPHEMA beads were produced by suspension polymerization of HEMA. DNA was then immobilized to the beads (4.4 mg/g), which resulted an anti-dsDNA antibody adsorption of 97.8 mg/g from SLE patient plasma. Successful desorption ratios (more than 98% of the adsorbed anti-dsDNA antibody) were achieved by using 1 M NaCl at pH 4.0 in acetate

buffer. It was possible to reuse these DNA-affinity beads without marked reduction in the adsorption capacities.

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