Heparin-Immobilized Poly(2-hydroxyethylmethacrylate)-Based Microspheres

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ABSTRACT: Poly(2-hydroxyethylmethacrylate) (PHEMA)-based microspheres (150–200 μm in diameter) were produced by a modified suspension polymerization of different type of comonomers—namely, acrylic acid, dimethylaminoethyl-methacrylate, and methylmethacrylate. These microspheres were activated with cyanogen bromide (CNBr) at pH 11.5, and heparin molecules were then immobilized through covalent bonds. The amount of immobilized heparin was controlled by changing the initial concentration of CNBr and heparin. The increase in the initial concentrations of both CNBr and heparin caused an increase in the amount of heparin immobilized onto microspheres for all polymer surfaces. The maximum heparin immobilization was observed on the PHEMA homopolymer microspheres (180 mg/g). The plain and heparin-immobilized microspheres were contacted with blood in *in vitro* systems and in *ex vivo* animal experiments. Loss of the blood cells and clotting times were followed. Anticoagulant effect of the immobilized heparin was clearly observed with blood coagulation experiments. Loss of cells in the blood contacting with heparin-immobilized microspheres was significantly lower than those observed with the plain microspheres. Bovine serum albumin adsorption onto the microspheres containing heparin on their surfaces was also studied. High albumin adsorption values (up to 127 mg/g) were observed in which the heparin-immobilized PHEMA microspheres were used.
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Key words: heparin; immobilization; poly(2-hdroxyethylmethacryalete) copolymers; microspheres

INTRODUCTION

A biomaterial is a substance used in medical devices or in prostheses designed for contact with the living body for an intended method of application and for an intended period. Synthetic polymers are the most diverse class of biomaterials. Polymeric biomaterials are widely used in both medical and pharmaceutical applications.[1] These applications include a variety of implants or other supporting materials (e.g., vascular grafts, artificial hearts, intraocular lenses, joints, mammary prostheses, and sutures), extracorporeal therapeutic and other supporting devices (e.g., hemodialysis, hemoperfusion, blood oxygenation and bags), controlled release systems (e.g., transdermal drug delivery patches, microspheres, and microcapsules), and clinical diagnostic assays (mainly as carriers).[2] All biomaterials must meet certain criteria and regulatory requirements before they can be qualified for use in medical applications. Depending on the intended end use, a biomaterial may be subjected to a set of tests, such as blood compatibility, tissue compatibility, carcinogenicity, cytotoxicity, mutagenicity, biodegradation, and mechanical stability.[3] Biomaterials in use come into contact with blood: First small molecules (e.g., water and ions) reach to the

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surface, which may or may not be adsorbed. This is followed by plasma protein adsorption. The first protein layer adsorbed on the biomaterial surface determines the subsequent events of the coagulation cascade (via the intrinsic pathway) and the complement activation (via the intrinsic and extrinsic pathways).[4]

Surfaces of polymeric biomaterials may be modified by using a variety of biological entities (e.g., bioactive substances, proteins, and cells) in order to increase their biocompatibilities. Chemical and physical modification of the polymeric surfaces may significantly increase their biocompatibilities. Biological modification may be considered as one further step to improve the biocompatibility. The main goal in this approach is to incorporate biological entities (e.g., heparin, urokinase, albumin) onto polymeric biomaterial surfaces to create biomaterial–biological system interfaces close to mother nature.[5]

Heparin is an important anticoagulant, used clinically to minimize thrombus formation on artificial surfaces. Heparin is an anionic linear polysaccharide chemically known as glycoaminoglycans. Heparin has the ability to combine with a large number of proteins. But heparin can sometimes cause undesirable acute (e.g., hemorrhage, anaphylaxis, and thrombocytopenia) and chronic (e.g., osteoporosis and hyperlipidemia) complications.[6] In order to eliminate these complications, numerous approaches have been tried, including minimal intermittent or regional heparinization, removal of excess heparin by using sorbents or immobilized heparinase, and immobilization of heparin onto blood contacting polymeric biomaterials.[7] Heparin and heparin–albumin conjugates have been adsorbed ionically and covalently to several biomaterial surfaces for improvement of blood compatibility.[8]

In our previous studies, we have immobilized several bioligands such as Protein A, low molecular weight heparin, DNA, collagen, and Cibacron Blue F3GA onto poly(2-hydroxyethylmethacrylate) (PHEMA) microspheres for different biomedical applications.[9–14] In this study, we attempted to prepare heparin-carrying PHEMA-based microspheres. A series of microspheres were produced by using acrylic acid (AA), methylmethacrylate (MMA), and dimethylaminoethylmethacrylate (DMAEMA). Heparin immobilizations were arranged by changing the cyanogen bromide (CNBr) and heparin initial concentrations. The plain and heparin-immobilized microspheres were contacted with blood in *in vitro* systems and in *ex vivo* animal experiments. Clotting times (i.e., coagulation time, activated partial thromboplastin time, and prothrombin time) and loss of blood cells were followed. Bovine serum albumin (BSA) adsorption onto these heparin-immobilized microspheres from aqueous media was also studied. This article presents the data obtained in these experiments.

EXPERIMENTAL

Preparation of PHEMA-Based Microspheres

The monomers, 2-hydroxyethyl methacrylate, AA, and DMAEMA, were obtained from Sigma (St. Louis, MO, USA) and used as received. MMA was obtained from Rohm (Darmstadt, Germany). The crosslinker ethylene glycol dimethacrylate (EGDMA; Merck, Darmstadt, Germany) was used in the polymerization recipe. Initiator was 2,2′-azobisisobutyronitrile (AIBN) (Aldrich, Rockford IL, USA). The dispersion medium was a saturated aqueous solution of magnesium oxide (4.0 g MgO/400 mL water, Sigma). PHEMA-based microspheres were prepared by a suspension polymerization technique.[15] The monomer phase including HEMA (24.0 mL) and comonomer (10 mL), EGDMA (12 mL), and AIBN (40 mg), was added to the dispersion medium within a reactor. Polymerizations were carried out at 70°C for 3 h and then at 90°C for 1 h. After cooling, the microspheres were separated from the polymerization medium by filtration, and the residuals (e.g., monomer, MgO, etc.) were removed by a cleaning procedure given in detail elsewhere.[10] To explain the process briefly: microspheres were filled in a packed-bed column, and washing solutions (i.e., a dilute HCl solution, and a water–ethanol mixture) were recirculated through the system, which also includes an activated carbon column, until to be assured that the microspheres are clean. Purity of the microspheres was followed by observing the change of absorbance of the samples taken from the liquid phase in the medium.

The swelling behavior of PHEMA-based microspheres was determined in distilled water. Dry microspheres were placed into distilled water and kept at a constant temperature of 25 ± 0.5°C. Swollen microspheres were periodically removed and weighed by an electronic balance (Shimadzu, Japan, EB.280 ± 1.10^{-3} g). The water content of the swollen microspheres were calculated by using the following relationship:

$$\text{Swelling ratio } \% = [(\text{Weq} - \text{Wdry})/\text{Wdry}] \times 100 \quad (1)$$

where W_{eq} and W_{dry} are weights of microspheres before and after swelling, respectively.

CNBr Activation

Prior to activation process, PHEMA-based microspheres were kept in distilled water for about 24 h and washed on a glass filter with 0.5*M* NaCl solution and water in order to remove impurities. Cyanogen bromide (CNBr, St. Louis MO, Sigma) aqueous solutions (100 mL) with different initial concentrations (10–50 mg CNBr/mL distilled water) were prepared. The pH of this solution was quickly adjusted to 11.5 with 2*M* NaOH while it was magnetically stirred (at 50 rpm). One gram of dry microspheres was then added to this solution and the activation procedure was continued for 60 min at a constant pH of 11.5. After the activation reaction, in order to remove the excess activation agent, the microspheres were washed with 0.1*M* $NaHCO_3$ and any remaining active groups (e.g., isourea) on the surfaces were blocked by treatment with ethanol amine (pH 9.1) and $FeCl_3$ solution (5% w/v) for 1 h. Then, the CNBr-activated microspheres were washed four times with distilled water containing 0.5*M* NaCl.

Heparin Immobilization

Heparin (Liquemine, 5000 IU/mL) was purchased from Roche (Switzerland). One gram of the freshly CNBr-activated microspheres was magnetically stirred (at 50 rpm) at a constant temperature of 25°C for about 2 h (i.e., equilibrium time) with 100 mL of a heparin solution. In order to observe the effect of the CNBr initial concentrations on immobilization of heparin, the CNBr initial concentration was varied between 10–50 mg/mL. The medium pH was 7.4 (0.1*M* phosphate buffer, K_2HPO_4-KH_2PO_4). Heparin was covalently bound to the CNBr-activated polymeric microspheres through a coupling reaction between the free hydroxyl or amine groups on heparin and the imidocarbonate groups on the activated microspheres. To obtain the effect of the heparin concentration on immobilization, the initial concentration of heparin was varied between 1 and 8 mg/mL. Then, the heparin-immobilized microspheres were washed with 0.1*M* $NaHCO_3$ and distilled water. The amount of heparin immobilization on the CNBr-activated microspheres was determined by measuring the decrease of heparin concentration and also by considering the heparin molecules adsorbed nonspecifically (the amount of heparin adsorbed on the plain microspheres), by the toluidine blue colorimetric method.[16]

Anticoagulant Activity of Immobilized Heparin

Coagulation Time (CT)

Heparin-immobilized microspheres 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 pooled human plasma (0.1 mL) was preheated to 37°C for 2 min, and then a 100 mg of microsphere was added into this medium and mixed immediately. The clotting time was measured by using the fibrometer method.[17]

Activated Partial Thromboplastin Time (APTT)

Heparin-immobilized microspheres 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 pooled human plasma (0.1 mL) was preheated to 37°C for 2 min. The partial thromboplastin (0.3 mL, bioMerieux, Marcy-l'Etoile, France) was also preheated to 37°C for 2 min and was added to preheated human plasma. Then,100 mg of microsphere was added into this medium. Thirty seconds later $CaCl_2$ (0.1 mL, 0.025 M) was added; then, the APTT was determined by using the fibrometer method.[18]

Prothrombin Time (PT)

In order to determine PT, the one-stage prothrombin method was used.[19] Heparin-immobilized microspheres were incubated in 0.1*M* phosphate buffer solution (pH 7.4) for 24 h at room temperature. Fresh frozen pooled human plasma (0.1 mL) was preheated to 37°C for 2 min. The thromboplastin (0.2 mL, bioMerieux, Marcy-l'Etoile, France) was also preheated to 37°C for 2 min and was added to preheated human plasma. Then a 100 mg of polymer microspheres was 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 by using fibrometer method.

Albumin Adsorption Studies

Adsorption of BSA (lyophilized, Fraction V, Sigma, USA) on the heparin-immobilized micro-

Table I Some Characteristics of PHEMA-Based Microspheres[a]

Microsphere	Swelling Ratio (%)	Nonspecific Adsorption (mg Heparin/g)
PHEMA	47.5 ± 0.5	3.9 ± 0.5
P(HEMA–AA)	41.7 ± 0.6	3.5 ± 0.8
P(HEMA–DMAEMA)	38.6 ± 0.4	12.6 ± 1.1
P(HEMA–MMA)	31.6 ± 0.7	4.3 ± 0.6

[a] Each experiment was repeated three times.

spheres was studied in batch system. The pH of the adsorption medium was adjusted to 7.4 by using 0.1*M* phosphate buffer (K_2HPO_4-KH_2PO_4). The initial concentration of BSA in the aqueous phase was varied between 1.0 and 8.0 mg/mL. Ionic strength of the adsorption medium was 0.01 (adjusted by NaCl, 0.01*M*). In a typical adsorption experiment, BSA was dissolved in 25 mL of buffer solution containing NaCl and microspheres (100 mg) were added. The adsorption experiments were carried out for 2 h (i.e., equilibrium time) at 25°C at a stirring rate of 100 rpm. At the end of the equilibrium period, the microspheres were separated from the solution. The BSA adsorption capacity was determined by measuring difference in the initial and the final concentrations of BSA within the adsorption medium spectrophotometrically at 280 nm.

Ex Vivo Animal Studies

An adult dog (20 kg weight) was used as the test animal in this study. The dog was anesthetized with nembutal. Then AV shunt was inserted into the right femoral artery and left femoral vein of test animal. Blood (heparinized, 500 IU/kg) was pumped to the two parallel hemoperfusion columns (with an internal diameter of 1.0 cm and height of 3 cm) containing heparin-immobilized microspheres. It should be noted that prior to the blood recirculation, the hemoperfusion columns were washed with 0.1*M* KCl in buffer until no further impurities (monitored by the absorbance at 280 nm) was detected in the washing solution. Blood flow rate was maintained at 2 mL/min by a blood pump. Blood samples were withdrawn from the inlet and outlet of the hemoperfusion column, and the platelet and leukocyte count of samples were determined.

RESULTS

PHEMA-Based Microspheres

Swelling ratios of the PHEMA-based microspheres are given in Table I. The maximum equilibrium content (i.e., swelling ratio) was 47.5%, which was for the PHEMA microspheres. Incorporation of comonomer (i.e., AA, DMAEMA, and MMA) within the polymer structure led to low swelling ratios. As expected, the P(HEMA–MMA) microspheres have the minimum swelling ratio (31.6%), because MMA is the least polar comonomer.

Untreated PHEMA-based microspheres were incubated with heparin solutions (heparin initial concentration: 2.0 mg/mL; temperature: 25°C; pH 7.4). The nonspecific heparin adsorption values (adsorption on the untreated microspheres) are also given in Table I. The nonspecific adsorptions for the PHEMA, P(HEMA–MMA), and P(HEMA–AA) were rather low and close to each other. For P(HEMA–DMAEMA) microspheres, the adsorption was about three times larger than the others, perhaps due to strong electrostatic interactions between positively charged amino groups of the microspheres and negatively charged heparin molecules.

Heparin Immobilization by Covalent Bonding

In order to optimize heparin immobilization onto the PHEMA-based microspheres, the effects of initial concentrations of CNBr (i.e., the activating agent) and heparin on the immobilization were investigated in batch adsorption-equilibrium studies. The initial concentration of CNBr in the activation medium was changed between 10 and 50 mg/mL in order to change the number of the activated sites on the polymer surfaces. These microspheres with different activation degrees were then incubated with heparin aqueous solutions (heparin initial concentration: 2.0 mg/mL; temperature: 25°C; pH 7.4).

Figure 1 shows the effects of the initial CNBr concentration on heparin immobilization. As seen here, the behavior of heparin immobilization on different surfaces was similar. Heparin immobilization capacities were increased with increasing the initial concentration of CNBr, up to 30 mg/mL. However, above this value, the effect was less pro-

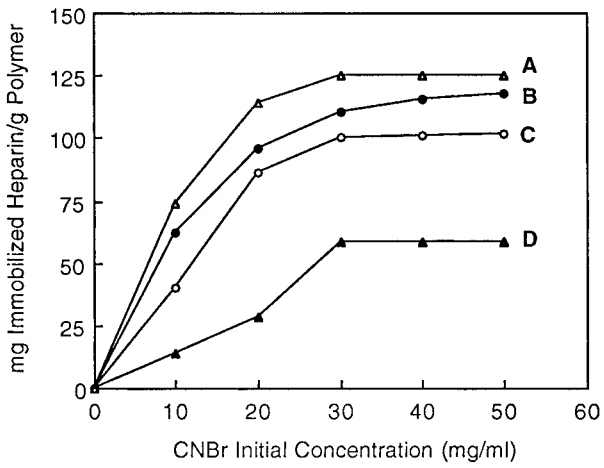

Figure 1 Effect of CNBr initial concentration on heparin immobilization: (A) PHEMA, (B) P(HEMA–AA), (C) P(HEMA–DMAEMA), and (D) P(HEMA–MMA). Heparin initial concentration: 2 mg/mL; Temperature: 25°C; pH 7.4.

nounced or even plateau values were achieved. The maximum heparin adsorption capacity was observed on the PHEMA homopolymer microspheres. Incorporation of the respective comonomers into the polymer structure causes a decrease in the relative amount of the —OH groups, which are activated by CNBr, and heparin molecules are then immobilized. The maximum heparin adsorption capacities of the CNBr-activated microspheres were 125, 117.8, 102, and 58.4 mg/g for PHEMA, P(HEMA–AA), P(HEMA–DMAEMA) and P(HEMA–MMA), respectively. Therefore, the order of heparin adsorption capacity as follows: PHEMA > P(HEMA–AA) > P(HEMA–DMAEMA) > P(HEMA–MMA).

Note that an increase in the CNBr initial concentration corresponds to a larger number of activated sites on the surface of the carrier. Therefore, as expected, higher amounts of heparin are coupled on the CNBr-activated polymer microspheres with higher number of activated sites. It should be noted also that there is always a saturation capacity that depends on the number of functional groups on the matrix and the size of the ligand molecules. However, the key factor in the performance of ligands immobilized on a solid surface is the ligand mobility after coupling rather than the total number of ligands available for coupling. Binding the heparin molecule tightly to the surface from multiple interactions would certainly reduce effective utilization of active sites on the heparin molecule.

In order to establish the effects of initial concentration of heparin, the heparin initial concentration was changed between 1 and 8 mg/mL. CNBr concentration in the activation medium was kept constant at 30 mg/mL. The microspheres with same activation degrees were then incubated with heparin aqueous solutions (temperature: 25°C; pH 7.4). Figure 2 shows the amount of covalently coupled heparin molecules on microspheres. As can be seen from Figure 2, immobilization capacities were increased with increasing heparin initial concentration in the incubation medium for all microspheres. The increase in the amount of immobilized heparin was less pronounced or even zero above 6 mg heparin/mL solution.

As can be seen here, the behavior of heparin binding on different polymer surfaces were quite different. For instance, for the PHEMA microspheres heparin immobilization capacity was maximum. In the case of negatively charged P(HEMA–AA), positively charged P(HEMA–DMAEMA), and relatively hydrophobic P(HEMA–MMA) surfaces, there were less heparin adsorption capacities.

Anticoagulant Activity of Immobilized Heparin

In order to estimate the anticoagulant activity of immobilized heparin, the *in vitro* CT, APTT, and PT tests were carried out with the plain and heparin-immobilized microspheres. It should be mentioned that the APTT test exhibits the bioactivity of intrinsic blood coagulation factors and the PT test relates to extrinsic blood coagulation

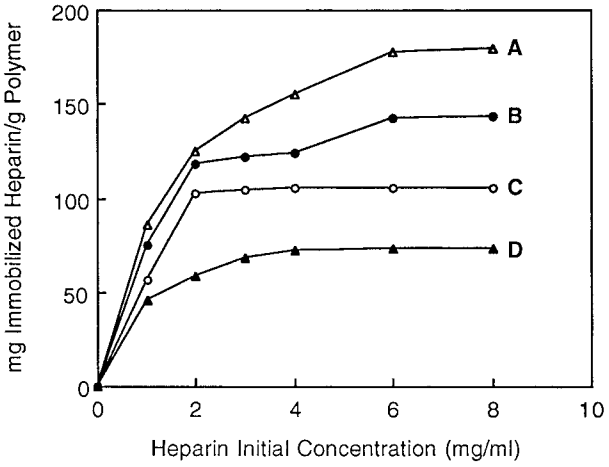

Figure 2 Effect of heparin initial concentration on heparin immobilization. (A) PHEMA, (B) P(HEMA–AA), (C) P(HEMA–DMAEMA), and (D) P(HEMA–MMA). CNBr initial concentration: 30 mg/mL; Temperature: 25°C; pH 7.4.

Table II Coagulation Times[a]

Microsphere	APTT (s)	PT (s)	CT (s)
PHEMA	40.8 ± 0.5	14.3 ± 0.3	235 ± 10.6
PHEMA/heparin[b]	49.4 ± 0.7	18.7 ± 0.4	347 ± 11.5
P(HEMA–AA)	38.9 ± 0.4	13.4 ± 0.3	228 ± 10.1
P(HEMA–AA)/heparin[c]	47.6 ± 0.6	16.5 ± 0.5	327 ± 9.7
P(HEMA–MMA)	37.7 ± 0.2	12.6 ± 0.4	220 ± 7.5
P(HEMA–MMA)/heparin[d]	43.6 ± 0.4	16.7 ± 0.2	317 ± 12.7
P(HEMA–DMAEMA)	38.7 ± 0.5	13.1 ± 0.1	229 ± 11.4
P(HEMA–DMAEMA)/heparin[e]	45.5 ± 0.3	16.9 ± 0.4	330 ± 8.6

[a] Each experiment was repeated three times.
[b] Heparin loading: 71.2 mg/g.
[c] Heparin loading: 65.4 mg/g.
[d] Heparin loading: 65.3 mg/g.
[e] Heparin loading: 58.4 mg/g.

factors on material surface. The CT test shows *in vitro* coagulation time. Table II summarizes the data obtained in these tests.

As can be seen from Table II, all the clotting times (CT, APTT, and PT) for the heparin-immobilized microspheres were significantly larger than those measured for the plain microspheres. Note that the CT, APTT, and PT values were taken after washing and drying of polymer microcarriers, but there were no differences that were observed previously. Therefore we concluded that the bioactivity of immobilized heparin was preserved during the immobilization procedure that we applied, and the activity was quite reproducible compared with the values reported in the related literature.[20,21]

Ex Vivo Animal Studies

In this part, the plain and heparin-immobilized polymer microspheres were used in an extracorporeal test system. An adult dog was used as the test animal. Two parallel perfusion columns were filled with 4.0 g of polymer microspheres and blood of dog was recirculated through the columns at 2.0 mL/min flow rate. Perfusion was carried out for 1 h. The blood samples were taken from the inlet and outlet of column in certain time intervals, and platelet and leukocyte counts were taken.

Table III summarizes hematological data obtained in *ex vivo* animal assay. Losses of platelet and leukocyte with heparin-immobilized micro-

Table III Platelet and Leukocyte Adhesion on Plain and Heparin-Immobilized Microspheres[a]

	Platelet ($\times 10^{-3}$/mm^3)		Leukocyte ($\times 10^{-3}$/mm^3)	
Microsphere	Initial/Final	Percent Loss	Initial/Final	Percent Loss
PHEMA	370/210	43.2	4.82/2.79	42.1
PHEMA/heparin[b]	365/300	17.8	4.75/3.86	18.7
P(HEMA–AA)	350/215	38.6	4.50/2.82	37.3
P(HEMA–AA)/heparin[c]	362/283	21.8	4.44/3.81	14.2
P(HEMA–MMA)	340/190	44.1	4.61/2.56	44.5
P(HEMA–MMA)/heparin[d]	354/265	25.1	4.78/3.65	23.6
P(HEMA–DMAEMA)	385/200	48.1	4.76/2.94	38.2
P(HEMA–DMAEMA)/heparin[e]	375/294	21.6	4.56/3.72	18.4

[a] Each experiment was repeated three times.
[b] Heparin loading: 71.2 mg/g.
[c] Heparin loading: 65.4 mg/g.
[d] Heparin loading: 65.3 mg/g.
[e] Heparin loading: 58.4 mg/g.

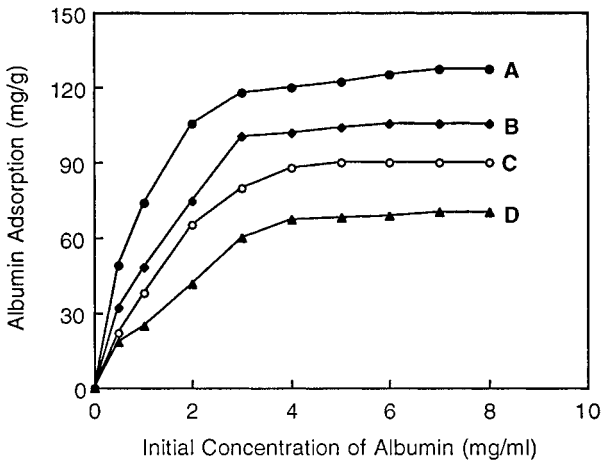

Figure 3 Effect of BSA initial concentration on BSA adsorption: (A) PHEMA, (B) P(HEMA–AA), (C) P(HEMA–DMAEMA), and (D) P(HEMA–MMA). Heparin loading: (A) 71.2 mg/g, (B) 65.4 mg/g, (C) 65.3 mg/g, and (D) 58.4 mg/g. Temperature: 25°C; pH 7.4.

spheres (between 17.8 and 25.1% for platelet adhesion and 14.2 and 23.6% for leukocyte adhesion) were lower than those observed with the plain polymer microspheres (38.6–48.1% for platelet adhesion and 38.2–44.5% for leukocyte adhesion). These observations allow the conclusion that heparin molecules were effectively immobilized by the procedure that we have applied in this study. Pressure drop in column applications is also important. In addition, the pressure drop values for all microspheres were measured and found to be around 30 mm Hg. As seen here, there is no significant pressure drop in the column.

BSA Adsorption from Aqueous Solution

Coating of biomaterial surface with biological molecules including heparin, urokinase, and brinolase have been experienced.[22–24] Among these, albumin has been found one of the most successful, which passivates the material surface and thus increases biocompatibility—in view of the fact that albumin coatings do not initiate blood coagulation, and also reduce platelet adhesion and consumption.

Figure 3 shows the effects of initial concentration of BSA on BSA adsorption onto heparin-immobilized microspheres. When the BSA concentration (i.e., the driving force for mass transfer) increased, the amount of BSA adsorbed onto PHEMA-based microspheres first increased and then reached an almost constant value. The maximum albumin adsorption capacities were 127 mg/g for PHEMA; 105 mg/g for P(HEMA–AA), 90 mg/g for P(HEMA–DMAEMA), and 70 mg/g for P(HEMA–MMA). The order of albumin adsorption capacity via heparin molecules was given as follows: PHEMA > P(HEMA–AA) > P(HEMA–DMAEMA) > P(HEMA–MMA).

CONCLUSION

Swellable PHEMA-based microspheres (150–200 μm in diameter) with varying water content were prepared, and the relationships between the surface chemical characteristics and blood compatibility were studied. Copolymer microspheres were activated with cyanogen bromide, and after heparin molecules were immobilized. The amount of immobilized heparin was controlled by changing the initial concentration of CNBr and heparin. The increase in the initial concentrations of both CNBr and heparin caused an increase in the amount of heparin immobilized onto microspheres for all polymer surfaces. The maximum heparin immobilization capacities of the CNBr-activated microspheres were 125, 117.8, 102, and 58.4 mg/g for PHEMA, P(HEMA–AA), P(HEMA–DMAEMA), and P(HEMA–MMA), respectively. Therefore, the order of heparin adsorption capacity was as follows: PHEMA > P(HEMA-AA) > P(HEMA-DMAEMA) > P(HEMA-MMA). The plain and heparin-immobilized microspheres were contacted with blood in *in vitro* systems and in *ex vivo* animal experiments. Lost of the blood cells and clotting times were followed. Anticoagulant effect of the immobilized heparin was clearly observed with blood coagulation experiments. All the blood-clotting times (CT, APTT, and PT) for the heparin-immobilized microspheres were significantly larger than those measured for the plain microspheres. Loss of cells in the blood contacting with heparin-immobilized microspheres was significantly lower than those observed with the plain microspheres. Bovine serum albumin adsorption onto the microspheres carrying heparin molecules on their surfaces was also studied. The maximum albumin adsorption capacities were 127 mg/g for PHEMA, 105 mg/g for P(HEMA–AA), 90 mg/g for P(HEMA–DMAEMA),and 70 mg/g for P(HEMA–MMA). The order of albumin adsorption capacity onto the heparin immobilized microspheres was PHEMA > P(HEMA–AA) > P(HEMA–DMAEMA) > P(HEMA–MMA). In conclusion, because of the good nonthrombogenic properties, the heparinized homo- and copolymers

seem to be very promising for biomedical applications.

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