Heparin-Coated Poly(hydroxyethyl methacrylate/albumin) Hydrogel Networks: *In Vitro* Hemocompatibility Evaluation for Vascular Biomaterials

Gülay Bayramoğlu,[1,2] Meltem Yılmaz,[1] Ertan Batislam,[3] M. Yakup Arıca[1]

[1]*Biochemical Processing and Biomaterial Research Laboratory, Faculty of Science, Kırıkkale University, 71450 Yahşihan–Kırıkkale, Turkey*
[2]*Department of Chemistry, Faculty of Sciences and Arts, Gazi University, 06500 Teknik Okullar, Ankara, Turkey*
[3]*School of Medicine, Department of Urology, Kırıkkale University, Kırıkkale, Turkey*

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ABSTRACT: Human serum albumin (AL) containing poly(hydroxyethyl methacrylate) (pHEMA; in tube form with an inner diameter of 6 mm) was synthesized for blood-contacting hydrogel networks via UV-initiated photopolymerization at 25°C. Tensile and breaking tests of pHEMA and pHEMA–AL-1–4 hydrogel networks were studied at their equilibrium water content. The mechanical strength of the hydrogel networks was found to be lowered by an increase in the ratio of AL in the polymer networks. To increase the blood compatibility and prevent thrombus formation, the surface of the pHEMA and pHEMA–AL-3 hydrogel compositions were coated with heparin (HEP). Contact-angle studies showed that the polarities (%) of the pHEMA–AL-3 and pHEMA–AL-3–HEP hydrogel networks were significantly increased in comparison with that of pure pHEMA. The fibrinogen adsorption and platelet adhesion were also reduced after the incorporation of AL and HEP into/onto hydrogel networks in comparison with the pure pHEMA control. Blood compatibility tests of the prepared hydrogel networks, which were intended to be used as blood-contacting materials, were examined with various parameters, such as the hemolytic activity, prothrombin time, activated thromboplastin time, and loss of blood cells in blood. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 109: 749–757, 2008

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INTRODUCTION

Polymers are used in biomedicine for the manufacturing of instruments for hemodialysis systems, extracorporeal circuits, heart valves, blood bypass tubes, prosthetic instruments, catheters, and medical devices coming into direct contact with human blood.[1–3] A series of complex interactions occur when blood comes into contact with an artificial surface. First, protein adsorption to the polymeric surface or cell (mostly platelet) adhesion occurs, and then activation of the blood coagulation system, fibrin accumulation, and clot formation occur.[4–7] The interaction between the biological environment (e.g., hard or soft tissue, blood, body liquids, or saliva) and biomaterials take place on the surface of the materials. The biological response of the living tissues against the biomaterials depends on the surface properties, such as the chemical composition, surface energy, resistance to corrosion, and tendency to denature of the neighboring proteins. Therefore, the surfaces of biomaterials are believed to play a vital role in determining biocompatibility.[8,9] The biocompatibility of a biomaterial is described with interactions on micrometer and nanometer scales between implants and biological systems. In addition, physicochemical surface properties of biomaterials, such as the chemical composition, wettability, surface energy, semiconductivity, and surface charge, play important roles in these interactions.[10–12] The logic of the surface modification of a biomaterial is to modify only the outer surface of the biomaterial and to protect the main physical properties. Thus, the mechanical properties and functions of the biomedical instruments are not affected, but a biomodification can be developed at the interface between the tissue and the material.[13] Therefore, the surface modification of synthetic polymeric materials in contact with blood is a new research area.[14,15] The latest research includes the grafting of biological macromolecules, such as heparin (HEP) and human serum albumin (AL), onto the surfaces of biomaterials developed for direct contact with blood with new surface modification methods.[16–24] HEP, which is an effective agent in decreasing thrombosis, is an anionic polysaccharide consisting of variably sulfated D-glucosamine and either L-iduronic or D-glucuronic acid.

Correspondence to: M. Y. Arıca (yakuparica@tnn.net).
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For example, Duncan et al.[17] prepared a heart valve with HEP entrapped on poly(hydroxyethyl methacrylate) (pHEMA) films and studied the antithrombogenic effect of entrapped HEP. Christensen et al.[12] grafted HEP onto the surface of a stent that was prepared as a vascular prosthesis and studied the effect of entrapped HEP on the platelets and complement activation system. In another study, HEP was immobilized on the surface of a polyurethane membrane with a hydrophilic poly(ethylene oxide) spacer arm, and the blood compatibility of this biomaterial was increased.[21]

The purpose of this study was to prepare blood-contacting materials from pHEMA and AL. To impede the formation of thrombus on the surfaces of pHEMA and pHEMA–AL-3 hydrogel networks, low-molecular-weight HEP was covalently immobilized on these hydrogels. The surface properties of these hydrogel networks were determined by the measurement of the contact-angle values against different test liquids, and the surface free energies of these hydrogels were calculated from contact angles with the acid–base method of van Oss.[25] The fibrinogen adsorption from an aqueous medium at different pH values, hemolytic activity tests, and the adherence of blood cells to the pHEMA, pHEMA–HEP, pHEMA–AL-3, and pHEMA–AL-3–HEP hydrogel networks were also studied.

EXPERIMENTAL

Materials

AL, fibrinogen, and low-molecular-weight HEP were purchased from Sigma Chemical Co. (St. Louis, MO). 2-Hydroxyethyl methacrylate (HEMA) and α-ά-azoisobutyronitrile (AIBN) were obtained from Fluka AG (Buchs, SC, Switzerland). Prothrombin time (PT) and activated partial thromboplastin time (aPTT) test kits were obtained from Teco Medical GmbH (Ergoldsbach, Germany). 1,1′-Carbonyl diimidazole (CDI) and other chemicals were analytical-grade and were purchased from Merck AG (Darmstadt, Germany).

Preparation of the pHEMA and pHEMA–AL hydrogel networks

pHEMA-based hydrogel networks were prepared via UV-initiated photopolymerization, as reported previously.[24,26] To check the effect of the AL concentration on the hydrogel network properties, four different AL concentrations were used in the polymer formulation mixture. The polymerization mixture (10 mL) contained HEMA (4.0 mL), AL (10–40 mg), AIBN (50 mg) as the polymerization initiator, and 6.0 mL of phosphate buffer (50 m*M*, pH 7.4). The resulting mixture was equilibrated at 25°C for 30 min in a water bath. It was then transferred into cylindrical glass molds lying inside one another. The open ends of the mold were closed with O rings. The pHEMA and pHEMA–AL-1–4 hydrogel networks were obtained (i.d. = 6 mm, length = 10 cm) with the mold. The synthesized tubes were removed from the mold and washed in a sonicated water bath in phosphate buffer (50 m*M*, pH 7.4).

Immobilization of HEP to the pHEMA and pHEMA–AL-3 hydrogel tubes

The surface of pHEMA and/or pHEMA–AL-3 hydrogel tubes was coated with low-molecular-weight HEP after the activation of CDI. The CDI activation was carried out in phosphate buffer (0.1*M* and pH 8.0, containing 2.0 mg/mL CDI) at 25°C for 24 h. After this period, the hydrogel tubes were removed from the solution and washed with phosphate buffer (50 m*M*, pH 7.4). Then, the activated pHEMA and/or pHEMA–AL-3 hydrogel tubes (0.5 cm length) were transferred into a HEP solution (2.0 mg/mL HEP in phosphate buffer: 0.1*M* and pH 8.0) and incubated at 22°C for 24 h. After this period, the pHEMA–HEP and pHEMA–AL-3–HEP hydrogel networks were transferred into fresh phosphate buffer and washed several times in a sonicated water bath.

Blood-compatibility studies

Protein adsorption

To determine the blood compatibility of the pHEMA, pHEMA–HEP, pHEMA–AL-3, and pHEMA–AL-3–HEP tubes, the adsorption of fibrinogen onto the hydrogel tubes was studied at 37°C and at different pH values (between 4.4 and 8.4) with a stirring rate of 100 rpm for 18 h in a batch system. The initial concentration of protein was 1.0 mg/mL in the individual adsorption medium. The initial and final concentrations of fibrinogen in the medium were measured at 280 nm with a double-beam ultraviolet–visible (UV–vis) spectrophotometer (model 1601, Shimadzu, Tokyo, Japan). Concentrations of proteins were changed between 0.05 and 3.00 mg/mL to prepare a calibration curve. The amount of adsorbed fibrinogen on the hydrogel tube surface was obtained with the following equation:

$$q = [(C_o - C)V]/S \qquad (1)$$

where q is the amount of protein adsorbed onto the hydrogel surface (μg/cm^2); C_o and C are the concentrations of the protein in the solution before and after adsorption, respectively (μg/mL); V is the volume of the protein solution (mL); and S is the surface area of the tubes (cm^2).

PT and aPTT

To perform PT and aPTT tests, the pHEMA, pHEMA–HEP, pHEMA–AL-3, and pHEMA–AL-3–HEP hydrogels were conditioned in a 0.85% NaCl solution as described previously. Human venous blood samples from a healthy volunteer were mixed with sodium citrate in a ratio of 1 : 9, and then plasma was obtained by centrifugation at 3000 rpm for 10 min. The hydrogel networks were contacted with 300 μL of citrated plasma, and they were incubated at 37°C for 1.0 h. As a control, plasma not in contact with hydrogel tubes was used.

For the determination of PT, 50 μL of PT-S reactive (Teco GmbH, New Fahsn, Germany) was added to 25-μL incubated plasma samples, and then they were incubated at 37°C; the coagulation times were determined with an automated blood coagulation analyzer in seconds. For the determination of the aPTT time, 25 μL of aPTT reactive (Teco) was added to 25-μL incubated plasma samples; then, they were incubated at 37°C for 5 min. The coagulation times were determined with an automated blood coagulation analyzer in seconds synchronously with the addition of a 2.5 m*M* calcium chloride solution (aPTT-P reagent, Teco) to the tubes.

Hemolytic activity

The pHEMA, pHEMA–HEP, pHEMA–AL-3, and pHEMA–AL-3–HEP hydrogel samples (0.5 cm long) were washed with a 0.85% NaCl solution for 24.0 h. Then, the hydrogel samples were incubated in whole human blood containing ethylene diamine tetraacetic acid at 37°C for 1.0 h, and the hydrogel-contacted blood in the tubes was centrifuged at 3000 rpm for 20 min. The amount of released hemoglobin from hemolysis red blood cells was measured with a UV–vis spectrophotometer at 545 nm.

Platelet adhesion

The hydrogel network samples were washed with physiological buffer solution, and 5 mL of blood (containing 347 × 10^3 cells/μL of platelets) was transferred to the test tubes. Blood was incubated with pHEMA, pHEMA–AL-3, and pHEMA–AL-3–HEP hydrogel networks at 37°C for 30 min. Before and after the incubation, the blood cells were analyzed with the complete blood count (CBC) method (Sysmex XT-2000i, Roche Diagnostics, Mannheim, Germany).

Characterization studies

Scanning electron microscopy (SEM)

The dried pHEMA, pHEMA–AL-3, and pHEMA–AL-3–HEP hydrogel samples were coated with a thin layer of gold under reduced pressure, and their scanning electron micrographs were obtained with a JEOL JSM 5600 scanning electron microscope (Tokyo, Japan).

Fourier transform infrared (FTIR) spectroscopy

FTIR spectra of the pHEMA, pHEMA–AL-3, and pHEMA–AL-3–HEP hydrogel networks were obtained with an FTIR spectrophotometer (1000 FTIR, Mattson, Bucks, England). The dry sample (ca. 0.1 g) was mixed with KBr (0.1 g) and pressed into a tablet form. The FTIR spectrum was then recorded.

Water content

The swelling properties of the hydrogel networks were determined at 25°C in a physiological salt solution (0.85% NaCl) with a gravimetric method as described previously.

Determination of the HEP content

The amount of immobilized HEP on the pHEMA–HEP and pHEMA–AL-3–HEP hydrogel networks was determined spectrophotometrically at 631 nm with the toluidine blue method.[14] The method is based on the determination of a decreasing amount of toluidine blue in the supernatant, which is adsorbed onto HEP. This procedure provides a simple analysis technique that allows a direct measurement of immobilized HEP. The amount of immobilized HEP on the hydrogel samples was calculated with the following equation:

$$\text{Amount of heparin } (\mu g)cm^{-2} = (C_i - C_f)/A \quad (2)$$

where A is the surface area of the polymers and C_i and C_f are the initial and final concentrations of HEP in the solution, respectively.

Mechanical testing

Mechanical properties of pHEMA, pHEMA–AL-3, and pHEMA–AL-3–HEP tube structures were determined from dog-bone shapes (20-mm span length, 3-mm width, and 0.65-mm thickness) cut from the hydrogel samples. The tensile and breaking experiments were studied with a Lloyd (Fareham Hampshire, England) LS500 mechanical testing machine at room temperature. Force–elongation curves were plotted at an extension rate of 2 mm/min.

Determination of the contact angles and surface free energies of the hydrogel networks

The contact angles of the hydrogels against different test liquids [i.e., water, glycerol, diiodomethane (DIM), ethylene glycol, and dimethyl sulfoxide (DMSO)] were measured by the sessile drop method at 25°C with a CAM 200 digital optical contact-angle meter (KSV Instruments, Ltd., Helsinki, Finland). Both the left and right contact angles and drop dimension parameters were automatically calculated from the digitalized image. The contact angle of each drop was measured as a function of time in 5-s intervals beginning at the instant at which contact was made between the liquid and substrate. The measurements were averages of five contact angles on at least three samples. The free surface energy parameters of the hydrogel networks were calculated with the contact-angle data of the probe liquids. The results were analyzed according to the acid–base method of van Oss.[25] In this method, the contact angles against at least three liquids with known values of γ^{LW}, γ^{+}, and γ^{-} are measured; γ represents the surface energy, and the superscripts LW, +, and − refer to the dispersive, Lewis acid, and Lewis base components, respectively. The values for each experiment are put into the following equation:

$$(1 + \cos\theta)\gamma_1 = 2[(\gamma_s^{LW} \times \gamma_s^{LW})^{1/2} + (\gamma_s^{+} \times \gamma_1^{-})^{1/2} + (\gamma_s^{-} \times \gamma_1^{+})^{1/2} \quad (3)$$

where θ is the contact angle, and l and s are liquid and solid, respectively. The total surface energy (γ^{TOT}) is regarded as the sum of the Lifschitz–van der Waals and Lewis acid and base components:

$$\gamma^{TOT} = \gamma^{LW} + \gamma^{AB} \quad (4)$$

where γ^{LW} designates the Lifschitz–van der Waals interaction, reflecting the long-range interactions and calculated from the measured DIM contact angles; γ^{AB} designates acid–base interactions such as hydrogen bonding; and γ^{+} and γ^{-} refer to the proton- and electron-donating character, respectively.

RESULTS AND DISCUSSION

Characterization studies

Equilibrium water contents of pHEMA, pHEMA–HEP, pHEMA–AL-1–4, and pHEMA–AL-3–HEP hydrogel networks (0.5 cm long) were determined in a phosphate buffer solution (pH 7.4; containing 0.85% NaCl) at room temperature, and the results are presented in Table I. It was observed that the equilibrium water content increased as the ratio of AL in the polymer increased. The reason for this

TABLE I
Equilibrium Water Contents of the Hydrogels

Sample	Equilibrium water content (%)
pHEMA	63.6 ± 2.1
pHEMA–AL-1	69.9 ± 1.2
pHEMA–AL-2	77.6 ± 2.4
pHEMA–AL-3	85.8 ± 1.7
pHEMA–AL-4	92.3 ± 0.9
pHEMA–HEP	71.2 ± 2.7
pHEMA–AL-3–HEP	99.8 ± 0.2

increase may be the presence of AL in the pHEMA network structure, which is much more hydrophilic than the pHEMA chains. Another reason for the increase in the water content of the AL-incorporated hydrogel may be the decrease in the chain length of the polymer with the addition of AL into the polymer network structure of pHEMA. As shown in Table I, the water contents of the HEP-immobilized hydrogel tubes were increased in comparison with the HEP-free counterpart. The increase in the water content may be due to the existence of different hydrophilic and charged groups on the HEP structures. These results indicated that the hydrophilicity of the pHEMA hydrogel networks was effectively improved by the incorporation of AL and HEP macromolecules into the pHEMA network structure.

Microstructures of polymer tubes (i.e., pHEMA, pHEMA–HEP, pHEMA–AL-3, and pHEMA–AL-3–HEP) were examined with SEM. The SEM micrographs showed significant differences in the surface morphology of the hydrogel networks. The SEM micrograph of the pHEMA hydrogel surface shows a very porous structure [Fig. 1(A)]. As shown in Figure 1(B), the pHEMA–AL-3 network pore dimensions were highly reduced as a result of the incorporation of AL molecules within the pHEMA networks. The surface of the pHEMA–HEP hydrogel networks, coated with HEP, exhibited a property of less porosity and smoothness with respect to the surface of pHEMA [Fig. 1(C)]. When the surface structure of pHEMA–AL-3–HEP networks was examined, the pores were either filled or closed, and the networks had a much smoother surface structure than other modified hydrogel networks [Fig. 1(D)].

FTIR spectra of pHEMA, pHEMA–AL-3, and pHEMA–AL-3–HEP were obtained and are presented in Figure 2. In the FTIR spectra of pHEMA–AL-3 and pHEMA polymers, a stretching vibration band at 3350 cm^{-1} due to the —OH group and an aliphatic stretching —OH band can be observed at 2950 cm^{-1}. In the FTIR spectrum of the pHEMA sample that did not contain AL, in the amide and amine peak region, no peak can be seen between 1700 and 1400 cm^{-1}, but two peaks can be observed at 1654 and 1569 cm^{-1} for the pHEMA–AL-3 compo-

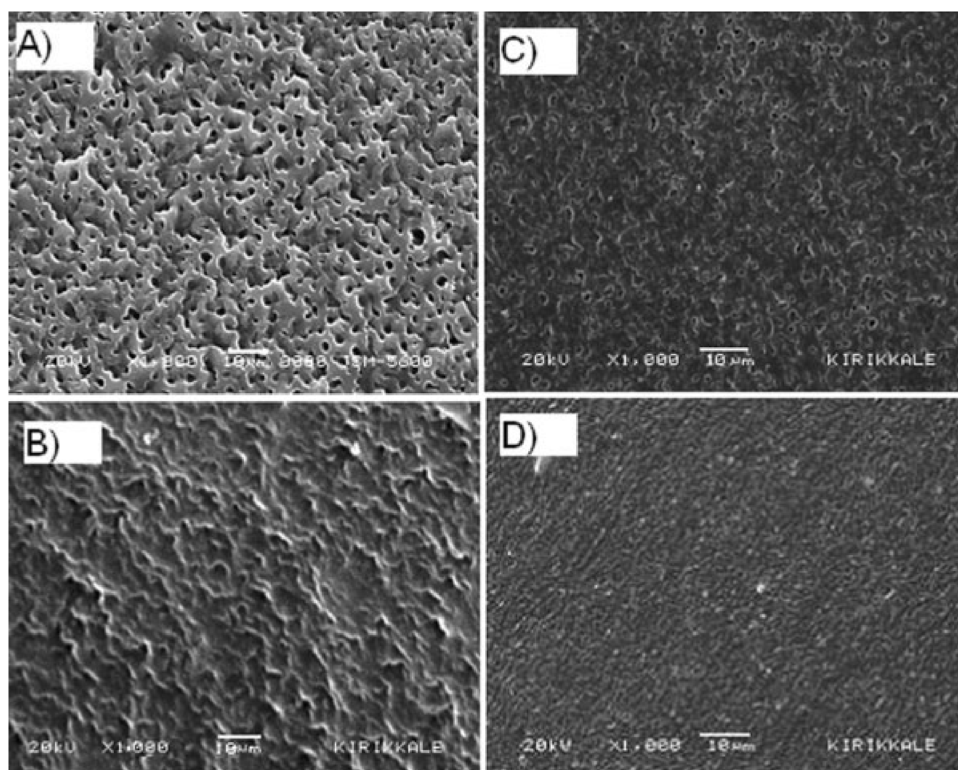

Figure 1 SEM micrographs at 1000× magnification: (A) pHEMA surface, (B) pHEMA–HEP surface, (C) pHEMA–AL-3 surface, and (D) pHEMA–AL-3–HEP.

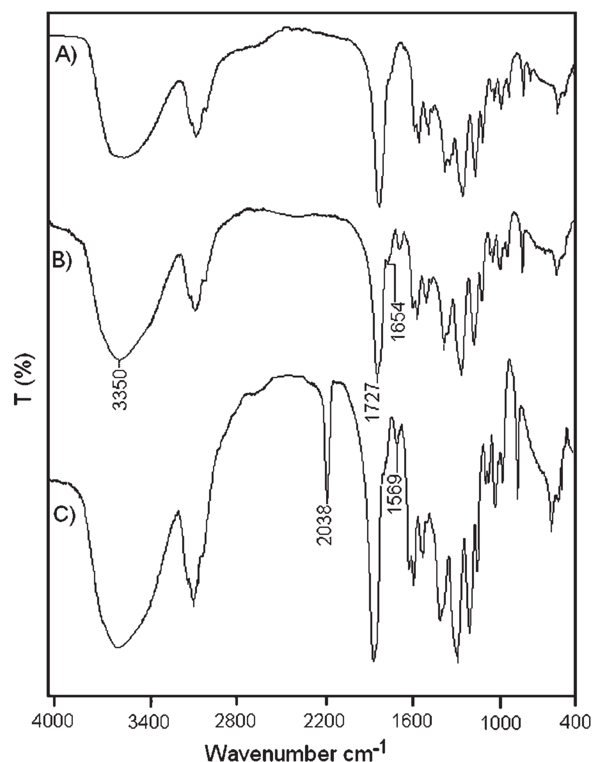

Figure 2 FTIR spectra of the structures of (A) PHEMA, (B) pHEMA–AL-3, and (C) pHEMA–AL-3–HEP.

sition containing AL. These peaks reflect a primer amine $—NH_2$ vibration, and it shows that AL is incorporated into the pHEMA networks. In the FTIR spectrum of the pHEMA–AL-3–HEP hydrogel, there exists a peak at 2038 cm^{-1} that cannot be observed for the pHEMA and pHEMA–AL-3 polymer networks. This peak comes from the sulfamate groups existing in the structure of HEP, and it shows that HEP was covalently immobilized to the surface of the polymer networks.

The antithrombogenic activity of the HEP layer was directly proportional to the amount of immobilized HEP. For example, Lindhout et al.[27] reported that the amount of immobilized HEP was changed between 0 and 35 $\mu g/cm^2$ on polyurethane membranes grafted with polyacrylamide, and the ratio of thrombin activation on the surface of the polymer to the HEP content (<2 $\mu g/cm^2$) decreased. They reported that the transfer limits of thrombin and the thrombin inactivation ratio were proportional to the HEP content of the material surface.[28] In our case, the amount of immobilized HEP on the surface of pHEMA and pHEMA–AL-3–HEP networks was found to be 23.8 and 78.6 $\mu g/cm^2$, respectively. As reported in the literature, the pHEMA–AL-3–HEP hydrogel network surfaces should have high antithrombogenic activity because of their high immobilized HEP content.

TABLE II
Mechanical Properties of the Polymeric Hydrogels

Sample	Tensile power (k/Pa)	Tensile breaking value (%)
pHEMA	167 ± 07	461 ± 12
pHEMA–AL-1	152 ± 05	384 ± 09
pHEMA–AL-2	126 ± 11	310 ± 12
pHEMA–AL-3	115 ± 05	271 ± 14
pHEMA–AL-4	98 ± 06	242 ± 11

Tensile and breaking tests of pHEMA and pHEMA–AL-1–4 hydrogel networks were studied at their equilibrium water content. As the loading of AL to the pHEMA structure increased, a decrease in the elastic module, depending on the amount of loaded AL, was observed (Table II). A 1.7-fold decrease in the power of the tensile strength (from 167 ± 07 to 98 ± 06 kPa) was achieved by the addition of AL (from 0 to 4 mg/mL) to the polymer preparation mixture. On the other hand, a 1.9-fold decrease in the breaking value of the tensile strength (from 461 ± 12 to 242 ± 11 kPa) was observed. These decreases could be due to the shortening of the chain length and lower crystallinity of the pHEMA networks associated with the increase in the AL content. It was observed that the AL-incorporated pHEMA networks, however, were less viscoelastic than that of pure HEMA because of the restrictions imposed by the macromolecular AL chains. Because the disruption of the crystallinity of the polymer networks was more extensive as the number of immobilized AL molecules increased in the pHEMA networks, the mechanical power of pHEMA was reduced as the AL content of the polymer networks increased.

The results for the contact-angle measurements of water, glycerol, DIM, ethylene glycol, and DMSO on the pHEMA, pHEMA–HEP, pHEMA–AL-3, and pHEMA–AL-3–HEP hydrogel surfaces are presented in Table III. In accordance with the Young equation, water has the highest contact-angle values, whereas DMSO has the lowest contact-angle values for all the tested hydrogel network samples. As shown in Table III, all the investigated samples yielded different contact-angle values. As expected, pHEMA has a polar surface and shows a water contact angle of 57.5°. After the incorporation of AL and/or HEP onto/into the hydrogel structure, the water contact angles decrease (Table III).

The determined overall surface free energy (γ^{TOT}), calculated with the acid base method of van Oss,[25] consisting of the sum of the Lifschitz–van der Waals (γ^{LW}) and acid–base components (γ^{AB}), applies to all the investigated samples at different values (Table IV). As shown in Table IV, the hydrogel networks seem to exhibit a slightly amphoteric character. However, the basic parameter (γ^{-}) is significantly larger than the acidic parameter (γ^{+}). The relatively high basic component of the surface energy (γ^{-}) is caused by the electron ion pairs of oxygen atoms contained in the hydrogel networks (hydroxyl, carbonyl, and carboxyl functionalities), which are effective in Lewis base sites.[29,30] It is interesting to observe that the γ^{AB} parameter of the pHEMA hydrogel network was significantly increased from 3.23 up to 8.26 mN/m^2 after the incorporation of AL and/or HEP into the network structure. Thus, all these parameters should be effective in determining the antifouling properties of the modified hydrogel networks when in contact with serum proteins and blood cells. It should be noted that the polarity percentage (i.e., water absorbance behavior) of the AL-incorporated and HEP-coated hydrogel networks significantly increased compared to that of pure pHEMA, as presented in Table IV; also, it has a trend similar to that of the γ^{AB} parameter because a more hydrophilic hydrogel structure could absorb water more easily.

Protein adsorption studies

AL has a property of thromboresistivity and therefore prevents thrombocytes from adhering to the surface of the biomaterials. Fibrinogen has the property of initiating the adherence of thrombocytes to the surface of a biomaterial. The antifouling properties of pHEMA–HEP, pHEMA–AL-3, and pHEMA–AL-3–HEP hydrogel networks were tested with fibri-

TABLE III
Contact Angles of pHEMA, pHEMA–HEP, pHEMA–AL-3, and pHEMA–AL-3–HEP Hydrogel Networks with Different Test Liquids

Test liquid	Contact angle (°)				
	pHEMA	pHEMA–HEP	pHEMA–AL-3	pHEMA–AL-3–HEP	γ_l
Water	58.5	54.7	45.9	45.1	71.3
Glycerol	56.2	53.5	43.8	42.2	64.0
DIM	35.8	40.2	40.3	41.9	50.8
Ethylene glycol	34.9	35.7	24.6	32.4	48.0
DMSO	31.6	18.8	15.0	13.4	44.0

TABLE IV
Surface Free Energy Parameters (mJ/m^2) of the Hydrogel Networks of pHEMA, pHEMA–HEP, pHEMA–AL-3, and pHEMA–AL-3–HEP According to the van Oss Method

Sample	γ^{LW} (mN/m^2)	γ^{+} (mN/m^2)	γ^{-} (mN/m^2)	γ^{AB} (mN/m^2)	γ^{TOT} (mN/m^2)	Polarity (%)
pHEMA	40.56	0.38	4.24	3.23	43.75	7.38
pHEMA–HEP	39.14	0.49	4.91	4.81	43.90	10.96
pHEMA–AL-3	38.56	0,74	5.59	8.26	46.74	17.67
pHEMA–AL-3–HEP	37.82	0.71	5.74	8.18	46.02	17.77

nogen in a batch mode with pure pHEMA as a control system. The pH of an adsorption medium has a significant influence on the amount of adsorbed protein. The hydrogel samples were incubated in the pH range of pH 4.4–5.4 in an acetate buffer and in the pH range of 6.4–8.4 in a phosphate buffer. The initial concentration of fibrinogen in the medium was 1.0 mg/mL, and the medium was stirred at 100 rpm at 37°C for 18 h. The fibrinogen reached its maximum adsorption values on the pHEMA, pHEMA–HEP, pHEMA–AL-3, and pHEMA–AL-3–HEP hydrogel networks at pH 4.4 after a 10-h incubation period and then remained constant after this time period. The amount of adsorbed fibrinogen on the hydrogel sample surface is presented in Figure 3. As shown in Figure 3, the pHEMA–HEP and pHEMA–AL-3–HEP polymers that were coated with HEP adsorbed less protein in comparison with the uncoated counterpart. Because the isoelectric point of fibrinogen was 5.5, the protein had a net negative charge at pH 7.4. On the other hand, the surface of the HEP-coated hydrogel networks was also negatively charged at a physiological pH value of 7.4 because the HEP molecules had several negatively charged functional SO_3^- and COO^- groups at the physiological pH value. As expected, at pH 7.4, the amount of adsorbed fibrinogen decreased upon the immobilization of HEP on the pHEMA and pHEMA–AL-3 hydrogel networks. The reduction in the adsorbed amount of fibrinogen was mainly due to the repulsion force between the protein and the coated HEP on the hydrogel networks; both had a negative charge. Thus, a decrease in the fibrinogen adsorption to pHEMA–HEP and pHEMA–AL-3–HEP polymer networks was observed. On the basis of these results, there was an improved biocompatibility response of the HEP-coated hydrogel networks in comparison with the HEP-free counterpart. It should be noted that HEP molecules remained biologically functional in this HEP immobilization method. These results were in good agreement with the related literature.[27,31–35] For example, Amiji[31] and Winterton et al.[32] studied the adsorption of plasma proteins (i.e., fibrinogen and AL) to HEP-immobilized polymeric surfaces, and they reported that fibrinogen and AL did not show any binding affinity to immobilized HEP at the physiological pH of 7.4.

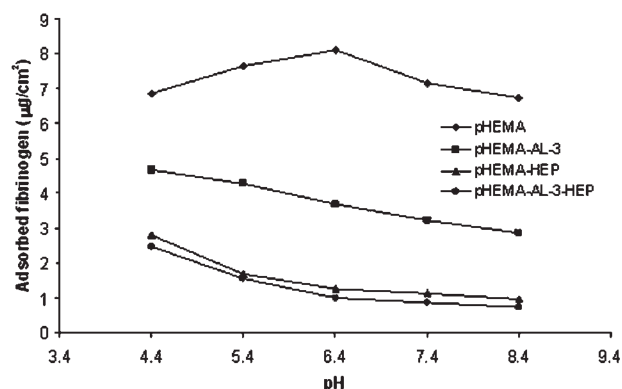

Figure 3 Amount of adsorbed fibrinogen in the pHEMA, pHEMA–HEP, pHEMA–AL-3, and pHEMA–AL-3–HEP hydrogel networks at different pH values at 37°C.

Blood compatibility studies

Determination of PT and aPTT

PT evaluates the factors found in the extrinsic and common pathways in the coagulation cascade. aPTT evaluates the intrinsic and common pathways. In the case of an absence of any coagulation factor in the extrinsic or common pathway, PT increases, whereas aPTT increases in the case of an absence of any coagulation factor in the intrinsic or common pathway. Within the scope of blood compatibility experiments of the hydrogel networks, the experiments of PT and aPTT were determined, and pHEMA, pHEMA–AL-3, and pHEMA–AL-3–HEP hydrogel networks were incubated with citrated plasma of a venous blood sample at 37°C for 1.0 h. Native plasma was used as a control. The durations of PT and aPTT obtained with the hydrogels of pHEMA, pHEMA–AL, and pHEMA–AL–HEP are presented in Table V. As shown in Table V, PT durations remained within normal ranges for all the tested samples. However, aPTT durations showed a rather high increase (3.57-fold) in comparison with the values of the control samples. The plasma incubated with pHEMA–AL-3–HEP hydrogels showed the highest aPTT value. It has been reported that immobilized HEP shows an antithrombogenic effect similar to the effect of free

TABLE V
PT and aPTT Durations for Contact with the Tested Samples

Sample	PT (10–14 s)	aPTT (20–40 s)
Control plasma	12.2	33.4
pHEMA	12.7	116.5
pHEMA–AL-3	12.3	115.3
pHEMA–AL-3–HEP	12.0	119.4

HEP in plasma. HEP catalyzes the interaction of various coagulation factors, especially of plasma proteins containing antithrombin (AT) III. It has been determined that HEP activates AT III when HEP-immobilized biomaterials come into contact with blood plasma.

Hemolytic activity

The hemolytic activity was studied with a spectrophotometric method at 545 nm. pHEMA, pHEMA–HEP, pHEMA–AL-3, and pHEMA–AL-3–HEP hydrogel networks were incubated with human blood. As a control, a blood sample was incubated in an isotonic solution under the same conditions. The ratio of released hemoglobulin from the red blood cells was accepted as 100% for blood incubated in distilled water. As shown in Table VI, the incorporation of AL and HEP into the pHEMA hydrogel structures resulted in a decrease in the hemolytic activity. When pure pHEMA was introduced into the blood sample, 0.95% hemolysis was observed. By the use of the pHEMA–AL-3–HEP composition, even this low degree of hemolysis was further decreased to 0.27%. As expected, a HEP layer on the hydrogel surface significantly decreased the red blood cell damage.

Cell adhesion studies

When a material comes into contact with blood, the blood proteins are first adsorbed onto the biomaterial surface and then denatured according to the force applied by the surfaces. Then, platelets interact with the deformed proteins and form aggregates. Platelets therefore are known to play a major role in thrombus formation.[25] For this reason, a study on platelet adhesion to determine the biocompatibility of a biomaterial is contemplated. CBC was performed for adherence testing of platelets. All obtained values, before and after contact with pHEMA–AL-3–HEP, were within normal ranges; thus, the adhesion of platelets onto pHEMA–AL-3–HEP hydrogel networks can be prevented by the HEP layers. The number of platelets before incubation slightly decreased in comparison with the number of platelets after incubation with pHEMA and pHEMA–AL-3 hydrogel networks. The decrease in the numbers of platelets may have come from the immobilization of them onto the polymer network surfaces. The decrease ratios for pHEMA and pHEMA–AL-3 polymer networks were 7.2 and 1.3%, respectively, and the numbers of adhering platelets were 39 and 7 platelets/mm^2, respectively. The increase in the platelet adhesion (%) on pHEMA was more pronounced in comparison with pHEMA–AL-3 networks. It should be noted that the incorporation of AL into the pHEMA structure significantly reduced the adhesion of platelets in comparison with pure pHEMA hydrogel networks. A similar observation was reported by Kang et al.,[36] who reported that platelet adhesion was significantly prevented by the immobilization of HEP onto polyurethane membrane surfaces.

CONCLUSIONS

Poly(2-hydroxyethyl methacrylate) hydrogel has been used in several biomedical applications such as contact lenses and soft tissue replacement. In this work, AL containing pHEMA hydrogel networks was prepared via UV-initiated photopolymerization, and some of the hydrogel network surfaces were coated with HEP. The water content of the pHEMA-based hydrogel increased with an increasing concentration of AL in the initial polymerization composition. The properties of pHEMA, pHEMA–HEP, pHEMA–AL-3, and pHEMA–AL-3–HEP hydrogel networks were examined for blood compatibility in terms of the PT, aPTT, hemolytic activity, platelet adhesion, and protein adsorption. The protein adsorption and platelet adhesion onto the surfaces of pHEMA and pHEMA–AL-3–HEP were reduced after coating with HEP in comparison with HEP-free counterparts. These results indicated that the biocompatibility, antifouling properties with respect to fibrinogen, and reduction in cell adhesion to the hydrogel networks were significantly improved after the immobilization of HEP on the hydrogel networks. All the modified pHEMA hydrogel networks showed improved blood compatibility in comparison with pure pHEMA. HEP-

TABLE VI
Hemolysis Values of the pHEMA, pHEMA–HEP, pHEMA–AL-3, and pHEMA–AL-3–HEP Hydrogels

Sample	Hemolysis (%)
Distilled water	100 ± 0.0
pHEMA	0.95 ± 0.2
pHEMA–HEP	0.53 ± 0.3
pHEMA–AL-3	0.78 ± 0.4
pHEMA–AL-3–HEP	0.27 ± 0.2

coated pHEMA–AL hydrogel networks have the potential to be used in blood-contacting devices such as blood bypass tubes because of their highly improved blood compatibility.

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