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Surface Modification of Polystyrene with the Bovine Serum Albumin–Tween 80 Complex and a Forecast of Biocompatibility

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Abstract—The surface of polystyrene was modified with the bovine serum albumin–Tween 80 complex. The adsorption of the complex and the formation of films on the surface of polystyrene were studied using the piezoelectric weighing method. The state of the modified surface was evaluated by contact angle measurements. The stability of the modifying layer was determined based on the critical interfacial energy values of the surface equilibrated with water. A conclusion was drawn that the complex can be effectively used to enhance the biocompatibility of polymer materials.

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Polymer materials are widely used in biomedicine for the manufacture of implants (organs, vessels, and tissues of synthetic materials) and for other purposes (the manufacture of membranes, catheters, hemodialyzers, blood storage containers, biosensors, drug transfer containers, etc.). In all cases, these materials should be biologically inert and biocompatible.

The results of studies performed over a period of many years [1–3] allow us to recognize both the general problems of biocompatibility and special problems related to the particular conditions of the use of biomaterials. Thus, for example, a material for the replacement of blood vessels should primarily be blood-compatible; this implies the low adhesion of blood cell structures (thrombocytes, leucocytes, macrophages, etc.) to the surface of the implanted material. The adhesion of thrombocytes provokes thrombosis. It is well known that, in contact between a polymer material and blood, plasma proteins are adsorbed on the surface of this material [4, 5]. In this case, the adsorption of serum albumin is even favorable for blood compatibility [6, 7], whereas the adsorption of fibrinogen enhances the adhesion of thrombocytes to induce complement activation, which is the initial stage of thrombosis [8, 9]. Note that serum albumin adsorbed on the surface of a material should retain its native conformation; otherwise, the blood compatibility of the material is dramatically impaired [4, 6]. Moreover, pathogenic bacteria, which are responsible for inflammatory processes that accompany implantation, should not adhere to the material [10]. An increase in the adsorption of serum albumins, especially, in a denatured state, also enhances the adhesion of bacteria. Thus, thrombogenicity and a probability of inflammatory processes are the main complications in the implantation of artificial materials contacting with blood [11].

Materials for medical purposes are tested with the use of a number of appropriate biological procedures in both in vitro and in vivo studies. However, historically (and this approach remains of current importance), the materials have been chosen based on studying their mechanical and surface properties. The contact angles of water (inflow wetting angles θ_a) have been used to characterize the surface properties (in almost all of the studies). It has been found experimentally that the surfaces should be hydrophilic to a certain measure. A correlation between the contact angle and the biocompatibility of a material [7, 10, 12–17] allowed one to find that, to a first approximation, the material is suitable for medical uses if the contact angles of water drops on the surface are ~60°. In this context, various techniques for the hydrophilization (increasing the wettability) of initially hydrophobic polymer surfaces have received wide acceptance [7, 10–25]. Among them are surface modification with lipids [10, 13, 16, 19, 20] and heparin [18, 21]; the grafting of hydrophilic monomers to the surface [22]; and partial surface oxidation with the formation of C–O, C=O, and O–C–O bonds [17]. The modification of polymer surfaces with polyethylene glycols has been most widely used in the past few years [7, 12, 14, 15, 23, 24].

An improvement in the biocompatibility of materials upon surface modification with polyethylene glycols was considered in a monograph [25]. However, the contact angle given only roughly indicates the biocompatibility because wetting effects can depend on surface microheterogeneity (nanotopography [14]) and roughness [14, 26], porosity [27], and the structure and volume of a modifying layer [28].

The problem of choosing medicinal materials in terms of mechanical surface stability in biological media is also solved based on wettability measure-

ments. In this case, the hysteresis of contact angles (difference between the inflow and outflow wetting angles θ_a and θ_r, respectively) is measured. The contact angle hysteresis $\Delta\theta = \theta_a - \theta_r$ suggests a change in the surface properties of the material. In this manner, Kurian et al. [12] found a membrane surface reorganization under changes in a contact medium (air or water). Depending on the concentration of polyethylene glycol, the inflow wetting angle (θ_a) changed from 96° to 126°, whereas the outflow wetting angle (θ_r) changed from 20° to 60°; this fact suggests an increase in the fraction of polyethylene glycol on the surface in an aqueous environment. Ruckenstein and Lee [29], who developed the criteria of biocompatibility, found that the behavior of a polymer in a biological medium depends on the polymer–water interface energy (σ_{SL}). For biocompatible materials, the value of σ_{SL} should be close to interfacial tension at the cell–water boundary. At the same time, the polymer should not exhibit a noticeable decrease in strength in contact with the solvent to avoid its dispersion into a liquid medium. According to Ruckenstein and Lee [29], surface energy components, particularly, the dispersion components of the surface energy of a solid, are independent of the nature of a nonpolar medium; that is, they are equal for both all hydrocarbons and air. Polar components can be affected during a long contact of a polymer with a polar phase. The following two equilibrium values are of paramount importance for characterizing the surface of a solid: the surface energy of the polymer in a nonpolar environment (σ_{SO}) and the surface energy of the polymer in a polar environment (σ_{SW}). Ruckenstein and Lee [29] reported a procedure for determining σ_{SL} based on the wetting method and made a criterial evaluation of blood compatibility ($\sigma_{SL} \approx 3$ mJ/m^2).

Based on the above, we conclude that polymers modified with serum albumin and protein–nonionic surfactant complexes, in which the surfactant contains oxyethyl chains (by analogy with polyethylene glycol), can be very promising biocompatible materials.

This work was devoted to a study of the applicability of these surfaces as biocompatible materials. For this study, we chose the polystyrene–bovine serum albumin (BSA; molecular weight M = 67000) model system and the polystyrene–(BSA–Tween 80 complex) system. Tween 80 is polyoxyethylenesorbitan monooleate with the average degree of oxyethylation n = 20 and M = 1308. The interaction of Tween 80 with BSA was studied in detail [30, 31]. Initially, the adsorption of BSA, Tween 80, and the BSA–Tween 80 complex on the surface of polystyrene was studied using piezoelectric microweighing [32]. This was required for quantitatively evaluating the polystyrene surface coverage with a modifying layer. The test concentration range of aqueous Tween 80 solutions was 10^{-7}–10^{-2} mol/l,* and the concentration of BSA (C_{BSA}) was 10^{-4} or 10^{-5} mol/l. The BSA–Tween 80 complex (1 : 1) was studied in the concentration region 10^{-7}–10^{-5} mol/l. The narrow range of the test complex concentrations ($C_{complex}$) resulted from the fact that, at $C_{complex} > 10^{-5}$ mol/l, the resulting complex was water insoluble and phase separation was observed.

Piezoelectric microweighing is a direct method for adsorption measurements; it is based on the dependence of the vibration frequency (f) of a quartz resonator (a microbalance sensor) on the amount (m) of substance applied to its surface [33]. From the change in the vibration frequency Δf (Hz), the adsorption Γ is calculated using the equation

$$\Gamma = \Delta m/S = -\Delta f/C_f, \quad (1)$$

where Δm is the weight of the substance adsorbed on the resonator surface; C_f is the mass sensitivity factor, which depends on the properties of piezoelectric quartz; and S is the working surface area of the resonator. In this study, we used AT-cut quartz resonators with silver electrodes and a natural frequency (f_0 = 5 MHz), for which $C_f = 2.27 \times 10^{-6}$ and $f_0^2 = 56.75 \times 10^6$ Hz cm^2 g^{-1} [32]. The adsorption Γ (g/cm^2) was calculated from the equation

$$\Gamma = -1.76 \times 10^{-8} \Delta f. \quad (2)$$

In the piezoelectric microweighing method, an adsorbent is either sputtered or applied as a thin film onto the sensor surface. Polymer films are usually supported from an organic solvent (polystyrene films were supported from a 0.5% solution of the polymer in toluene). The film thickness was 200 nm. In accordance with the Ruckenstein procedure, the polar and dispersion components of the interfacial tension of a solid surface equilibrated with water ($\sigma_{SW(W)}$) were calculated based on the measurements of inflow and outflow wetting angles and under conditions of selective wetting. Under certain assumptions, this value is determined by the equation

$$\sigma_{SW(W)} = \{(\sigma_W^p)^{1/2} - (\sigma_{SW}^p)^{1/2}\}^2 + \{\sigma_W^d\}^{1/2} - (\sigma_{SW}^d)^{1/2}\}^2, \quad (3)$$

where σ_W^p and σ_W^d are the polar and dispersion components of the surface tension of water, respectively; σ_{SW}^p and σ_{SW}^d are the polar and dispersion components of interfacial tension at the solid–water interface, respectively. The latter two values were determined from the outflow wetting angles of water upon delivering an air bubble (θ_{VW}) and an octane drop (θ_{OW}) to the sample surface, which was preliminary kept in water for 12 h and placed in water. The calculation was performed using the following equations:

*The critical micelle concentration (CMC) of Tween 80 is $CMC_{Tween\ 80} = 1.4 \times 10^{-4}$ mol/l).

$$\sigma_{SW}^{p} = (-\sigma_{OW}\cos\theta_{OW} + \sigma_{W} - \sigma_{O})^{2}/4\sigma_{W}^{p}; \quad (4)$$

$$\sigma_{SW}^{d} = (\sigma_{OW}\cos\theta_{OW} - \sigma_{W}\cos\theta_{VW} + \sigma_{O})^{2}/4\sigma_{O}. \quad (5)$$

The octane–water interfacial tension (σ_{OW}) was 21.8 mJ/m^2 (Table 1 summarizes the energy characteristics of water and octane).

To modify polystyrene films with aqueous solutions of BSA, Tween 80, and the BSA–Tween 80 complex, the samples were exposed to the corresponding solutions for 30 min; then, they were washed with water and dried. Before contact angle measurements, the films were kept in water for 12 h.

The adsorption of Tween 80 on polystyrene dramatically increased at $c >$ CMC (Fig. 1); this fact was usually attributed to the adsorption of micelles on a solid surface [34]. In the monolayer coverage of polystyrene, the surface area (S_m) per Tween 80 molecule in the adsorption layer is 66 nm^2, which is close to the landing area of the Tween 80 molecule in a saturated monolayer at the water–air interface [35]. The values of BSA adsorption on polystyrene are higher than the well-known published data; this is due to the formation of a protein polylayer on the surface [27]. After washing the samples modified with the protein in water, the values of adsorption consistent with published data were obtained (Fig. 2) because only the first protein layer, which is tightly bound to the polystyrene surface, remained on the surface. The adsorption of the complex increased with concentration (as the concentration of Tween 80 in the mixture was increased), and this increase was most sharply pronounced at $c_{BSA} = 10^{-4}$ mol/l. It is likely that the adsorption of the complex stimulated protein coprecipitation on the surface with an excess of the protein in a mixed solution.

The calculated values of $\sigma_{SW(W)}$ for the test surfaces suggest that the modification of polystyrene with BSA solutions did not afford sufficiently low (~3 mJ/m^2) interfacial energies (Table 2). In the modification of polystyrene with Tween 80 solutions, the interfacial energy was $\sigma_{SW(W)} \approx 0.25$–$1.3$ mJ/m^2, which implies the dispersion of the substrate into a liquid phase and the degradation of the material.

Table 1. Energy characteristics of liquids used for the calculation of $\sigma_{SW(W)}$

Liquid	σ_L	σ_L^p	σ_L^d
Water	72.6	50.8	21.8
Octane	21.8	–	21.8

However, good results were obtained in solutions with a certain concentration of the BSA–Tween 80 complex; this allowed us to use these modifying layers for providing biocompatibility. The polystyrene surfaces modified with the BSA–Tween 80 complex (1 : 1) at a concentration of $4 \times (10^{-7}$–$10^{-5})$ mol/l exhibited the inflow wetting angles (θ_a) equal to 53°–58°, which is consistent with the above correlation data. Consequently, they are potentially biocompatible materials. The interfacial energies of surfaces equilibrated with water (2.4–6.4 mJ/m^2) are also consistent with the critical values of biocompatible materials, which remain stable in contact with biological media. Note that the surface properties of polystyrene modified with BSA–Tween 80 complexes depend only slightly on the structure and composition of the modifying layer. Lin et al. [36] used an analogous approach in a study of the effect of the surface properties of polyurethanes on their biocompatibility. The polar and dispersion components of surface tension and the interfacial energy were calculated in accordance with a published procedure [37]. However, Lin et al. [36] did not keep polyurethane samples in water for a long time. The estimated interfacial energies of porous membranes (1.14–0.10 mJ/m^2) suggest the instability of the material in an aqueous

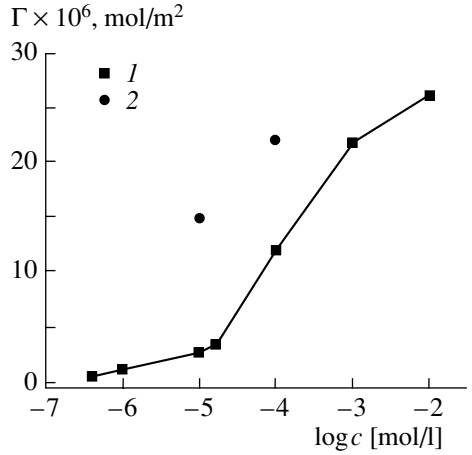

Fig. 1. Adsorption of (*1*) Tween 80 and (*2*) BSA on polystyrene from aqueous solutions.

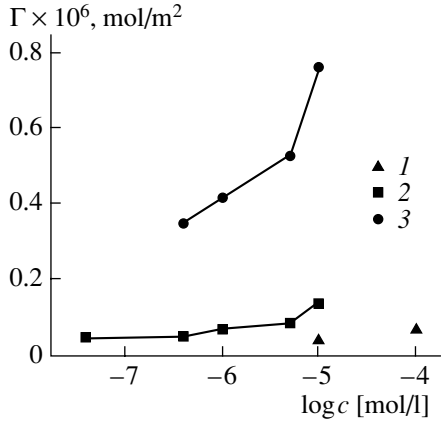

Fig. 2. Adsorption of (*1*) BSA and (*2*) the Tween 80–BSA complex on polystyrene from aqueous solutions.

Table 2. Interfacial surface–water energy $\Gamma_{SW(W)}$

Surface state	Contact angles, deg			Interfacial energy $\sigma_{SW(W)}$, mJ/m^2
	θ_a	θ_{VW}	θ_{OW}	
Unmodified polystyrene	86	80	146	18.8
Modified polystyrene				
Modifier				
BSA				
c = 10^{-5} mol/l	70	37	150	15.4
$c = 10^{-4}$ mol/l	70	30	122	9.7
Tween 80				
$c = 4 \times 10^{-7}$ mol/l	60	70	148	0.25
$c = 3 \times 10^{-5}$ mol/l	58	66	153	1.16
$c = 8 \times 10^{-5}$ mol/l	52	65	163	1.3
$c = 1.1 \times 10^{-4}$ mol/l	52	70	172	1.04
BSA–Tween 80 complex*				
$c = 4 \times 10^{-8}$ mol/l	63	40	155	8.8
$c = 5 \times 10^{-6}$ mol/l	66	58	110	6.4
$c = 10^{-5}$ mol/l	65	56	148	3.84
BSA–Tween 80 complex**				
$c = 4 \times 10^{-7}$ mol/l	61	53	150	5.9
$c = 10^{-5}$ mol/l	62	58	134	2.4

*$c_{BSA} = 10^{-5}$ mol/l.
**$c_{BSA} = 10^{-4}$ mol/l.

medium at satisfactory biocompatibility evaluated in a number of biological tests.

Similar values of contact angles and interfacial energies for polystyrene surfaces modified with the BSA–Tween 80 complex in systems with various values of c_{BSA} in a mixed solution provide support to the conclusion that the 1 : 1 complex is formed, whose concentration depends on the concentration of the deficient component Tween 80 [31].

Note that the contact angle hysteresis $\Delta\theta = \theta_a - \theta_{VW}$ reached a minimum for the surface of polystyrene ($\Delta\theta = 6°$), whereas it was 10°–12° for the surface modified with the solutions of the complex. This was due to a structural rearrangement in modifying layers at the solid–water interface. This was also supported by a change in the interfacial energy upon surface modification with the BSA–Tween 80 complex (from 8.8 to 2.4 mJ/m^2). Low interfacial energies upon surface modification with Tween 80 (from 1.3 to 0.25 mJ/m^2) suggest that the nonionic surfactant is a strong modifier of the polystyrene surface; however, the modifying film is unstable in contact with an aqueous medium. Pluronics, whose films are stable in water, can be very promising for the modification of polymer surfaces [38]. The surface modification of polystyrene with BSA–Tween 80 complexes results in the simultaneous presence of BSA and Tween 80, which bears oxyethylene chains, on the surface of the hydrophobic polymer. These results indicate that, in principle, the biocompatibility of a material can be improved, and they form the basis for the subsequent biological tests, in particular, the adsorption of fibrinogen on modified samples.

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