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Communications to the Editor

Design of Stimuli-Responsive Surfaces Prepared by Surface Segregation of Polypeptide-*b*-polystyrene Diblock Copolymers

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Since materials interact with their environment through the interface, surface modifications are required to adapt them for applications as superhydrophobicity[1] to improve biocompatibility or to minimize energy losses and wear under shear. The preparation of sophisticated materials with responsive surfaces having the ability of reversibly change back and forth between two contradictory comportments has drawn considerable attention in recent years. For instance, so-called adaptive or active surfaces with dual hydrophobic−hydrophilic behavior have been prepared with polymer brushes that respond either/both to pH or/and to temperature.[2] In this contribution we used polypeptides as stimuli-responsive segments. The polypeptide secondary structure can be finely and reversibly tuned between α-helical, *β*-sheet, or random coil depending on the environmental conditions (pH, temperature, ionic strength, etc.). In addition, poly(amino acid) thin films deposited on inorganic surfaces have been recently highlighted for their technological potential interest in advanced materials such as biosensors, optical switches, or liquid crystal displays.[3]

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To appropriately modify polymer surfaces, chemists usually resort to chemical or physical treatments such as flame, low-temperature plasma treatments, or electron beam irradiation. The functional groups or radicals present at the surface are thus used, in an additional step, to covalently anchor polymer chains. Two main approaches are known to immobilize polymer chains to a solid surface, namely the "grafting to" method, where preformed chains are bonded by a chemical reaction to surface reactive groups,[4] and the "grafting from" method, based on the initiation of the monomer polymerization from the surface.[5] However, controlled functionalization of polymer surfaces still remains challenging. The above-mentioned methods of surface modification enclose several important drawbacks such as poor control of the chemical composition[6] or mechanical mismatch of the outmost layer that can lead in some cases to delamination.[7]

A possible alternative path to those modification methods involves the segregation of one of the components of a polymer blend to the surface. The preferential enrichment at the surface level is caused by differences in both entropy[8] and surface energy of the blend components;[9] a large surface excess of chain ends for materials with low surface energy groups (e.g., fluorocarbons) or the surface depletion of higher energy chain ends (e.g., carboxylic or amine groups) at the air−polymer interface evidence the significance of the enthalpy of the polymeric additive.[10] Since surface segregation is a spontaneous process, the surface structures obtained are at the thermodynamic equilibrium. In addition, segregation occurs in situ without any further processing, allowing the manufacture of self-healing surfaces in which the chemical composition is recovered by further surface segregation if the intial surface is damaged.

In this contribution we describe the preparation of stimuli-responsive surfaces containing polypeptide poly(L-glutamic acid) (PGA) segments at the surface by using amphiphilic polystyrene-*b*-poly(L-glutamic acid) block copolymers as surface modification agents. The surface composition long-term stability is assured by two main features: first, the high glass transition temperature of the polystyrene used as a matrix with very low chain mobility at room temperature. Second, since we are introducing segments rather than single functions, the system

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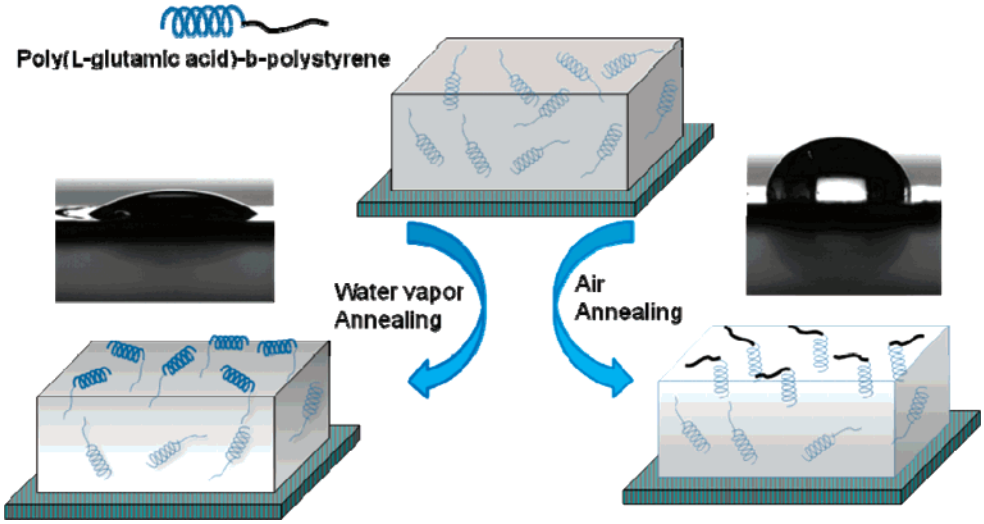

Figure 1. Scheme of a polymer blend film containing 20% of diblock copolymer PS_{27}-*b*-PGA_{20} either annealed to air (right) or annealed to water vapor (left).

shows microphase segregation at the surface between the two imcompatible blocks to a certain extent which makes it difficult for further rearrangement of the surface.

The synthesis of similar diblock copolymers has been already reported[11] and was carried out in several steps. First, styrene was polymerized by atom transfer radical polymerization (ATRP). By adequately tuning the conversion to values lower than 40%, a large percentage of end-brominated polystyrene groups should be obtained. The end-terminal bromo group was easily modified into an amine function by reaction with 1,4-diaminoethane.[12] The amine-modified PS was employed, in turn, as macroinitiator for the ring-opening polymerization of γ-benzyl ester-L-glutamate *N*-carboxyanhydride. Finally, deprotection under basic conditions (KOH/H_2O/THF) led to the amphiphilic PS-*b*-PGA diblock copolymers. The block copolymers have been characterized by ^{1}H NMR spectroscopy and GPC both to evaluate the integrity of the structure and to determine the chemical composition.

Polymer films of thickness greater than 300 nm were prepared by spin-coating from concentrated THF solutions (30 mg/mL) onto silicon substrates. Additive and matrix were codissolved at various blend concentrations: 5−30% of diblock copolymer and 95−70% of polystyrene (M_n = 600 000 g/mol). Relatively thick films were employed for two main reasons: to avoid dewetting problems that appear in thin polystyrene films above its glass transition temperature and to separate the support−polymer and the polymer−air interfaces in order to reduce the number of competing interactions that can alter the surface segregation phenomenon.

To achieve surface segregation, blend films prepared from PS_{27}-*b*-PGA_{20} were annealed in water vapor-saturated atmosphere for 36 h at 90 °C in order to modify the surface composition and to improve the reorientation of the hydrophilic polypeptide block at the interface (Figure 1). The characterization of the peptide-modified surfaces was carried out by both contact angle measurements and X-ray photoelectron spectroscopy (XPS). Water contact angle measurements indicated the surface enrichment of polypeptide segments as a consequence of the humidity treatment. Whereas the surfaces exposed to air exhibit water contact angles of 90°—as typically found in pure polystyrene films—the films treated in water vapor are rather hydrophilic, with contact angles below 50°.

X-ray photoelectron spectroscopy (XPS) measurements shown in Figure 2 evidenced important differences on the film surface

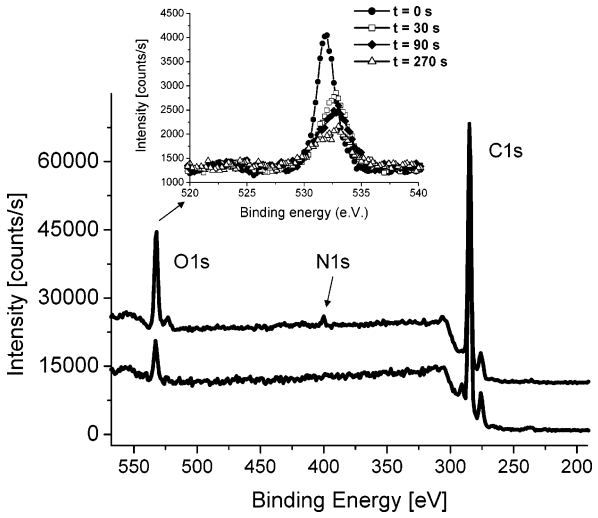

Figure 2. XPS spectra of a polymer blend film containing 20% of diblock copolymer PS_{27}-*b*-PGA_{20} not annealed (below) and annealed to water vapor (up). Inset: evolution of the O_{1s} signal after different sputtering times from 0, 30, 90, and 270 s for the water-annealed film. The significant decrease of the oxygen quantity after 30 s indicated the surface enrichment of the polypeptide block at the surface.

composition after annealing. Surface segregation upon annealing is indicated by the larger area of both N_{1s} (∼401 eV) and O_{1s} (∼532 eV) peaks, characteristic of poly(L-glutamic acid) block. This fact reveals the tendency of this block to migrate toward the interface under these annealing conditions. To further characterize the surface enrichment of the diblock copolymer, concentration depth profiles were obtained by sequential Ar^+ ion-beam sputtering of the polymer surface at different times (estimated sputtering rate 0.12 nm/s). The inset in Figure 2 shows the oxygen signal obtained for the water-annealed films after different sputtering times (0, 30, 90, 270 s). The oxygen peak decreased in intensity by about one-third of its original value after 30 s of sputtering. This result indicates the presence of an oxygen gradient as a function of depth and supports the surface enrichment on the polypeptidic segment. After longer sputtering times the oxygen signal remains constant at the average amount of polypeptide within the material.

Further information on the properties of the peptide modified surfaces was obtained by using the methylene blue method (Figure 3). This method uses a positively charged dye capable of interacting with the carboxylic functions in their negatively

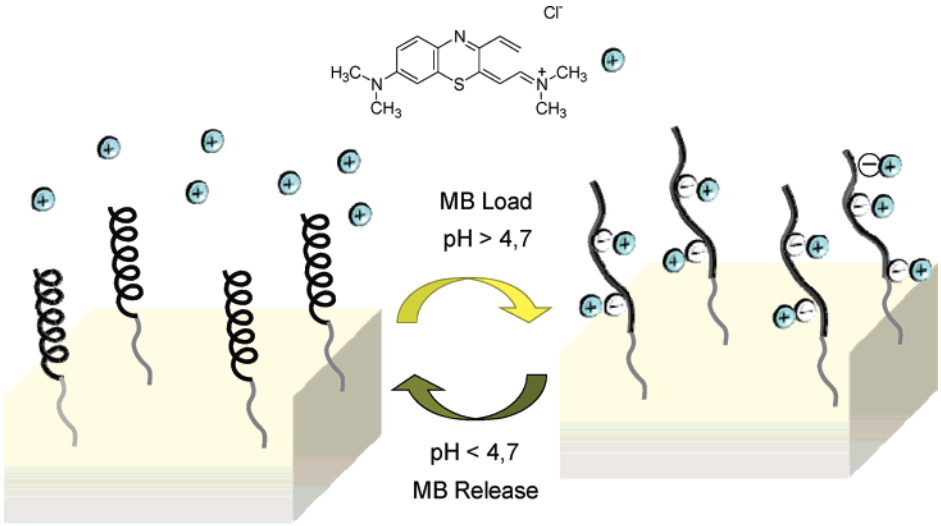

Figure 3. Scheme of the load and release approach of MB on the polypeptide-functionalized surfaces. At high pH values the carboxylic acid groups are negatively charged and are thus able to establish electrostatic interactions with MB. At low pH values, the carboxylic groups are neutralized and the MB is released.

charged form, i.e., at pH values above 5. The information obtained is twofold: first, we can estimate the amount of carboxylic groups revealed at the interface, since the methylene blue adsorbed can be released at lower pH values and thus be quantified by UV−vis spectroscopy (see Supporting Information). Second, given that the dye is adsorbed at high pH by electrostatic interactions and released under acidic conditions, this test constitutes a proof of the pH-responsive character of the polypeptide-enriched surfaces. Transitions between charged and neutralized states have been associated in synthetic polypeptides—particularly in tethered polypeptides—with changes in the secondary structure between α-helices (neutralized) and extended coiled chains (charged).[13] The water contact angle measurements carried out at different pH values (3 and 10) also confirmed the stimuli-responsive character of the prepared surface. The surfaces containing the polypeptide in its α-helical conformation are relatively hydrophobic, and the contact angle is significantly higher ($\theta = 80°$) than those surfaces having the polypeptide negatively charged (poly(L-glutamic acid)) ($\theta < 10°$). Such a large change of the surface wettability with pH have been previously observed in oxidized polyethylene surface and has been atributed to a larger hydrophilicity of the carboxylate anions.[14]

Atomic force microscopy (AFM) was also used to study the responsive character of the polypeptide-enriched surfaces by measuring the interaction force between a silicon nitride AFM tip and the substrates. This technique has been used in the past to study a broad range of surfaces, for the estimation of their surface charge[15] as well as the isoelectric point of organic and inorganic oxide surfaces. Figure 4 represents the interaction force profile between the Si_3N_4 AFM tip and the poly(L-glutamic acid)-functionalized surfaces either nontreated (a) or annealed to water vapor (b) measured under water at different pH values. In both cases, a long-range attractive interaction due to the van der Waals forces between the tip and the substrate can be identified at pH 3. For a sphere-flat system, this interaction can be described as $F = -A_{123}R/6D^2$,[16] where R is the tip radius, A_{123} is the Hamaker constant for the interaction of Si_3N_4−substrate through water, and D is the tip−substrate separation. By fitting the measured data to this equation (using the nominal fabricant values for the cantilever spring constant and the tip radius) a value of $A_{123} \approx 0.7 \times 10^{-19}$ J is obtained, in good agreement with values previously reported for similar systems.[17]

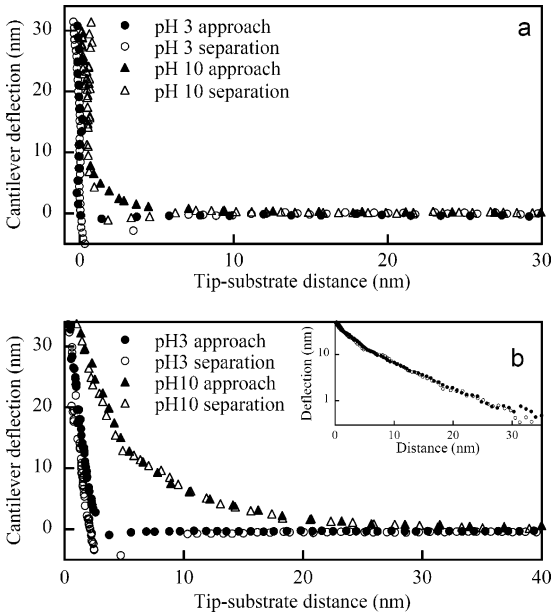

Figure 4. Normal tip−surface interaction force between the Si_3N_4 AFM tip and a nontreated surface (a) and a water-annealed surface (b) at different pH values. The semilog representation of the force measured for the water annealed surface (inset) illustrates the long-range exponential decay of the repulsive force.

On the opposite side, a long-range exponential repulsion, typical of the interaction between charged surfaces, is observed at pH 10. This exponentially decaying repulsion—as can be clearly observed in the semilog representation, inset of Figure 4b—is due to the similar sign electric charge of the AFM tip and the polymer surfaces. It is not related to the steric interaction between extended chains on the substrate and the tip: assuming an extended chain length of about 3.5 Å per residue,[18] the full polypeptide length will be 7 nm, clearly insufficient to account for the long range of the repulsion observed. As can be observed in the figure, this repulsion is significantly enhanced in the annealed samples. In addition, the electrostatic repulsion disappears at low pH values below the isoelectric point of Si_3N_4—where the tip is positively charged but the carboxylic acid groups are neutralized—illustrating the responsive character of the surfaces.

In addition to the long-range interaction forces, a short-range steric component of the force profile can be clearly observed at closer separations. While for the untreated case (Figure 4a) there is a step increase in repulsive force (a hard-wall repulsion), for the treated case (Figure 4b) the steric repulsive interaction displays a more gradual increase—probably related to the steric interaction between the peptide chains and the tip—before the abrupt hard-wall repulsive interaction is observed. These changes in the tip−subtrate interaction force clearly indicate the enrichment on poly(L-glutamic acid) of the surface after annealing under humid conditions.

In summary, we demonstrated that the surface segregation is an appropriate method to prepare functionalized surfaces with polypeptide segments. Poly(L-glutamic acid), revealed at the surface by annealing in humid environment, has the capability of changing back and forth between a charged and a neutralized state giving a pH-responsive character to the surface. Such surfaces are currently being evaluated for different potential applications including cellular adhesion in which both surface charge density and structure are key parameters.[19]

Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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