A New Gas Discharge Process for Preparation of Non-fouling Surfaces on Biomaterials

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Abstract: A non-fouling surface containing immobilized polyethylene oxide (PEO) was achieved using an argon radio-frequency glow discharge treatment (RFGD) of polyethylene films precoated with Brij hydrocarbon-PEO surfactants. Surface wettability of RFGD-treated and washed surfaces increased the most when PEO surfactants with unsaturated and/or long alkyl tails were used. ESCA measurements of treated and washed surfaces showed increases of surface O/C ratios and ether carbon peaks in high resolution Cls spectra. These results demonstrate the retention of the PEO surfactants on the treated surfaces. Fibrinogen adsorption on these treated surfaces was significantly reduced, from 500 to 50 ng/cm², indicating the non-fouling properties of the RFGD-immobilized PEO surfactants.

INTRODUCTION

Protein-repellent or 'non-fouling' surfaces are needed as components of drug delivery vehicles to avoid loss of high-cost peptide and protein drugs. They are also potentially useful as coatings for drug-containing nanoparticles, both to avoid phagocytosis in the liver and also to affect distribution of the particles in different organs. Such surfaces are also important to other biotechnological and medical applications, such as diagnostic assays, biosensors, bioseparations, implants and medical devices. The non-fouling properties of a wide variety of surface treatments have been investigated.¹ The deposition of compositions containing polyethylene oxide (PEO) has received the most attention.¹⁻⁸ It has been shown that when PEO chains of MW *c.* 1000 or more are immobilized on a surface, the adsorption of proteins as fibrinogen can be reduced significantly.

In this study, we have developed a novel radio-frequency glow discharge (RFGD) process to bind a PEO-containing surfactant onto a hydrophobic surface. In this process, the surfactant is first

'anchored' on the polymer surface via physical adsorption from a solvent which swells the substrate polymer. Then the solvent is evaporated and the adsorbed surfactant is bound covalently to the surface by a gas discharge treatment, which causes 'crosslinking' of the surfactant with the polymer molecules in the surface. In this paper we will present results obtained with a variety of PEO surfactants, physically adsorbed onto polyethylene surfaces and then bound to the surface with an argon gas discharge.

MATERIALS AND METHODS

Materials

Table 1 lists the code names and compositions of the compounds studied in this paper. PEO1K, Brij surfactants (from Sigma Chemical Co.) and Triton X-165 (as a gift from Rohm and Haas Co., Philadelphia, PA) were used as received. Brij99 was selected as a model compound for the adsorption and desorption studies. The low-density polyethylene

Table 1. PEO surfactants and PEO used in this study

PEO surfactant	Chemical structure ^a	Average molecular weight	Average PEO molecular weight	O/C ratio
Brij99	C _{18:1} -EO ₂₀	1148	880	0.36
Brij78	C ₁₈ -EO ₂₀	1150	880	0.36
Brij35	C ₁₂ -EO ₂₀	1066	880	0.40
Triton X-165	C ₈ Ph-EO ₁₆	910	700	0.37
PEO Homopolymer				
PEO1K	(CH ₂ CH ₂ O) ₂₃	1000	1000	0.50

^a Provided by the manufacturer.

(LDPE) films (Cadillac Plastic, Seattle, WA) were cleaned sequentially with CHCl₃, acetone and water for 15 min each in a sonicator and dried in air. Reagent-grade chemicals were used as received in this study without further purification. Fibrinogen was purified from fresh baboon blood (from the Regional Primate Research Center, Seattle, WA) and radio-labelled with ¹²⁵I according to previously published protocols.⁹

Adsorption and desorption of Brij99 on LDPE

Brij99 was chosen as a model surfactant for optimization of the adsorption and washing or desorption steps. A simple dip-coating method was applied to adsorb Brij99 onto LDPE films. LDPE films were dipped in a 1% w/v Brij99 solution, freshly prepared in chloroform, at room temperature for 30 s. Then the coated films were dried in a laminar flow hood for one day at room temperature. Desorption (or washing) of the surfactant from the coated but untreated surfaces was studied in both water and chloroform solvents. After RFGD treatment, treated films were washed with water for 30 min twice and soaked overnight in fresh water before further surface characterization. This process was designed to completely remove all of the physically adsorbed Brij99 from the surface.

Gas discharge treatment

A capacitive radio-frequency flow discharge at 13.56 MHz (HF-300, ENI Power Systems Inc., Rochester, NY) was used to treat LDPE or LDPE/PEO-surfactant surfaces in a glass cylinder reactor (i.d. 11.5 cm × 80 cm). A static argon (Air Products and Chemicals Inc., Allentown, PA, Pre-pure grade: > 99.95%) gas discharge without flow was generated at a reactor pressure of

25 μm Hg, a low power of 2.5 or 5 W, and ambient temperature.

Surface analyses

The RFGD-treated surfaces were characterized by ESCA and water contact-angle measurements. ESCA measurements were done on an SSX-100 spectrometer (Surface Science Instruments, Mountain View, CA) using a monochromatic AlKα X-ray source with a 5 eV floodgun. A Ramé-Hart goniometer was used to measure advancing-water contact angles of treated films at room temperature in air.

Fibrinogen adsorption

Polymer films (15 mm × 11 mm) were prehydrated in citrated phosphate buffered saline with 0.02% sodium azide and 0.01 M sodium iodide (CPBSzi) at 37 °C for 4 h. Then, an ¹²⁵I-labelled baboon fibrinogen solution was added to reach a 0.2 mg/ml total protein concentration (average counts = 2 × 10⁶ cpm/mg fibrinogen) and the films were incubated at 37 °C for 2 h. After incubation, the protein adsorbed films were eluted with 100 ml of fresh CPBSzi and counted with a γ-counter.

RESULTS AND DISCUSSION

ESCA was used to evaluate the effectiveness of the wash step (Fig. 1). Results indicate that the physically adsorbed Brij99 surfactant is easily removed from the untreated surface by soaking in either chloroform or water for 2 or 5 min, respectively. These results emphasize that simple physical adsorption of the surfactant, even in a swelling solvent such as CH₂Cl₂, is not sufficient to bind it to

Fig. 1. ESCA O/C ratio shown as a function of the kinetics of desorption (washing) time in water or chloroform for Brij99 physically adsorbed on untreated LDPE. Brij99 was deposited on LDPE surfaces from a 1% w/v chloroform solution for 30 s at room temperature, dried for one day and then washed at room temperature in either water or chloroform.

the LDPE surface. ESCA results of RFGD-treated and washed LDPE and LDPE/Brij99 surfaces are shown in Fig. 2. It can be seen that there is an increase of surface O/C ratios of RFGD-treated LDPE, indicating that the treatment causes some surface oxidation of LDPE, although the O/C ratios still remain significantly less than on the treated surfactant-LDPE films. The surface O/C ratios of RFGD-treated LDPE/Brij99 films increase with treatment time up to a plateau at 30 s, indicating the probable presence of PEO surfactant molecules on the treated surfaces when compared

to the control LDPE films. Thus, the treatment must bind the surfactant to the substrate surface. The recombination of alkyl and polymer free radicals, formed by the Ar RFGD treatment is the most likely explanation of such an immobilization, leading to crosslinking of the PEO surfactant to the polyethylene chains.

Advancing water-contact angles on the treated and washed control LDPE, LDPE/PEO-homopolymer and LDPE/PEO-surfactant films are shown in Fig. 3. The RFGD-treated LDPE surfaces exhibit a slight decrease in water contact

Fig. 2. ESCA O/C ratios for LDPE and LDPE/Brij99 surfaces after Ar RFGD treatment and washing in water.

Fig. 3. Water contact angles on Ar RFGD-treated LDPE and LDPE/PEO-surfactant surfaces after washing and drying.

angles, probably due to surface oxidation, as suggested by the ESCA results. Only surfaces modified with PEO surfactants containing a long unsaturated (Brij99) or saturated alkyl chain (Brij78), show a large reduction of water contact angle ($< 20^\circ$) for treatment times over about 45–90 s. No significant improvement of surface wettability was observed on the surfaces modified with the PEO homopolymer, PEO1K. It can also be seen that other PEO surfactants with a shorter alkyl group (Brij35) or an aryl group (Triton X-165) showed no improvement when compared with the LDPE control. These results support the proposed cross-

linking mechanism which should be favored by longer and unsaturated alkyl chains. It has been previously reported that polyethylene forms free radicals which cause surface crosslinking when PE is treated with inert gas plasmas.¹⁰ This process was named CASING (Crosslinking by Active Species of INert Gas). It is likely that the mechanism of the immobilization of PEO surfactants is similar.

Fibrinogen adsorption on RFGD-treated LDPE and LDPE/Brij99 surfaces is shown in Fig. 4. Fibrinogen adsorption on RFGD-treated and washed control LDPE films increases with increase

Fig. 4. Fibrinogen adsorption on Ar RFGD-treated LDPE and LDPE/Brij99 surfaces. Results are shown for 2.5 and 5 W plasma power levels. After prehydration of films in CPBSzi buffer for 4 h, protein adsorption was carried out in 0.2 mg/ml fibrinogen solutions at 37 °C for 2 h.

of treatment time as well as treatment power, probably because of the increased surface oxidation. On the other hand, the RFGD-treated LDPE/Brij99 surfaces exhibit a significant reduction of fibrinogen adsorption to about 50 ± 11 and $110 \pm 18 \text{ ng/cm}^2$ when treated 15 s with 2.5 and 5 W Ar plasmas, respectively. These results also support the retention of the Brij99 on the LDPE surface. When RFGD treatment time is prolonged, fibrinogen adsorption increases on the LDPE/Brij99 surfaces, which may be due to the surface oxidation and/or degradation of PEO due to the etching action of the argon plasma. In summary, the best non-fouling surface was obtained when LDPE/Brij99 films were treated at a low power level for a short treatment time, e.g. 2.5 W and 15–30 s. This is a very simple, clean and short process for producing a non-fouling surface.

CONCLUSIONS

A non-fouling polymer surface, exhibiting very low fibrinogen adsorption, was obtained using a short, low-power Ar RFGD treatment on an LDPE surface which had been precoated with a PEO surfactant. This simple process may have wide applicability for modifying polymer surfaces in general as well as biomaterial surfaces in particular.

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