Protein-binding topology formed on polypropylene during reaction-induced phase separation
Proteinbindung Topologie bildete sich auf Polypropylen während der Reaktion-induzierten Phasentrennung

Introduction

Established methods continue to be used to fine-tune the surface traits of low surface energy plastics such as polypropylene [1]. Nonetheless, more approaches are required to facilitate the engineering of mesoscale or nanoscale sized surface structures along plastics. In this study, the oxidative activation, surface engineering and protein-binding ability of modified polypropylene tubes was examined. Bovine serum albumin was used as test protein in view of the potential of human serum albumin to passivate plastic-coated medical products.

Summary: Subsequent to oxidation, the tubes proved chemically reactive and revealed a meso-structured, high-area surface. The new topology was caused by an oxidation-induced phase separation. These surfaces were treated with the hydrolysis products of aminopropyltri-ethoxysilane and ninhydrin, affording the corresponding amino and aldehyde derivatives. The performance of each tailored surface was assessed by the extent to which albumin could be loaded and retained following several washings. The successful use of oxidation to forge mesoscale topologies implied that other reactive chemical approaches might also be used to induce phase transformations. Such strategies, if developed, could provide alternatives to mesopattern problematic surfaces.

Materials and Methods

Eppendorf Safe-Lock brand tubes (2ml capacity) were obtained from Eppendorf GmbH. Dialysis bags (3500 MWCO) were obtained from Pharmacia. Bovine serum albumin, distilled water, solvents and reagents were obtained from Sigma-Aldrich Chemie GmbH.

Ammonium persulfate solution (1M, 1ml) was delivered so that each tube was filled to half capacity. Tubes were capped, heated (70ºC, 0-24h, no motion), washed with water and isopropanol, and dried under vacuum. For the control surfaces, ammonium sulfate (2M, 1ml) was used in place of persulfate.

Samples were dried in a vacuum oven prior to ATR-FTIR analysis. Clear, scuff-free regions were clamped along the ATR accessory of a Bruker model Equinox 55 infrared spectrophotometer. Twenty scans were averaged using OPUSV 3.1 software.

The tubes were left holding solution of the composition aminopropyltriethoxysilane/water/isopropanol (1:4:95 v/v/v), during which time (25°C, 1h) reactive silanol species formed *in situ* [2]. The tubes were decanted and washed with isopropanol. Aminopropylsilyl species along the surface were cured (70°C, 16h), presumably affording a multi-layered aminopropyl-siloxane-silanol hydrate surface. The tubes were flushed with water and dried. Control surfaces were treated similarly.

Accessible aminopropylsilyl moieties were deaminated to afford propanal moieties by reaction with propanolic ninhydrin (1% wt/v, 1.5ml, 60°C, 30min) [3]. All surfaces were washed extensively with water and ethanol, and dried. Native/ammonium sulfate control surfaces were also treated with ninhydrin. Surface-accessible amino groups, and by default, the expected aldehyde products, could be quantified (575nm) against known amounts of aminopropyltriethoxysilane in propanolic ninhydrin.

Scanning electron micrographs were obtained using a JEOL model JSM-6500F instrument with a beam voltage of 10-20kV. The tubes were coated with carbon.

Aliquots of trace-dansylated bovine serum albumin (1.5ml) were transferred into the tubes, centrifuged initially (30s), incubated under manual inversion (25°C, 2h), and discarded. The tubes were washed in salt solution (2ml 1M NaCl, 3min) under manual inversion and decanted. A second salt wash was performed. The tubes were agitated subsequently with water (3min) and decanted. Lastly, the tubes were incubated with 10% Triton X100 (2ml, 3min) and rinsed. Each surface was viewed and photographed under UV light after every work-up step.

Results and Discussion

Figure 1 shows the carbonyl region FT-IR spectra of polypropylene oxidized from 0h to 24h. To a first approximation, the signal increased proportionally with reaction time, affording a maximum signal after 16h. At least 3 overlapping signals were apparent, denoting 3 types of carbonyl products. While only carbonyl signals have been presented, signals were also noted in the functional group (4000-1300cm^{-1}), fingerprint (1300-900cm^{-1}) and low-frequency (900-600cm^{-1}) regions [4].

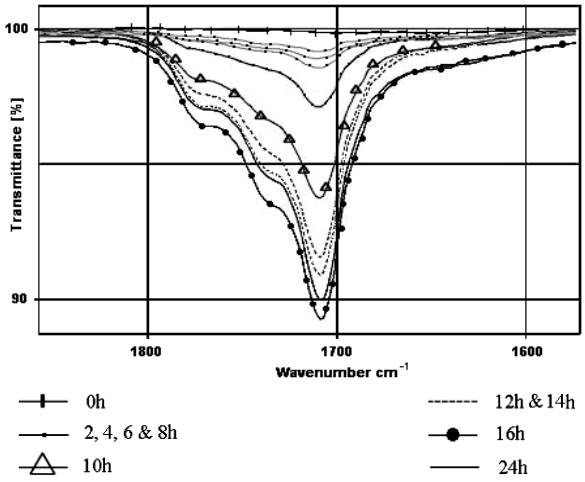

Fig. 1: The ATR-FTIR spectra illustrates a composite carbonyl signal buildup during the time course oxidation.

The SEM micrographs in figure 2 show the development of a mesoscale topology during the course of oxidizing injection-molded polypropylene tubes. No significant change of appearance was noted in the topology by 8h

oxidation time (A). On the other hand, minor changes had developed by 10h, in the form of sparsely distributed bulges of approximate 400nm diameter (B). A relatively brief period of dramatic change was noted thereafter, vis-à-vis the sponge-like mesoscale topology shown at 12h reaction time (C). Further reaction did not appear to alter the landscape (D).

Clearly, the sudden topological change in time (Fig. 2) did not reflect the time-course oxidation data (Fig. 1). This contrast of data proved that a phase separation was responsible for transforming the surface, as opposed to any constant & inwards-moving degradative process [5].

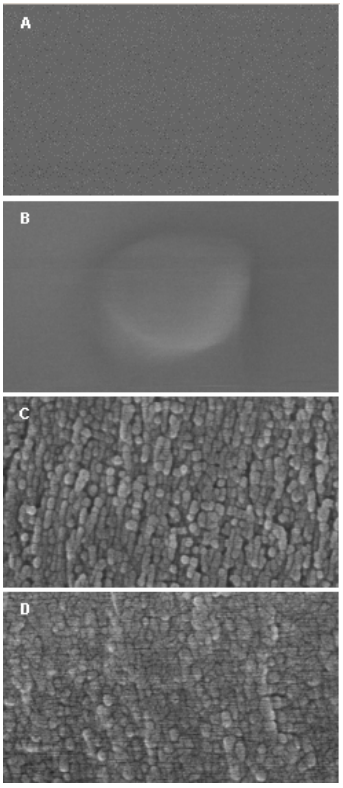

Fig. 2: Scanning electron micrographs of polypropylene oxidized for 8h (A), 10h (B), 12h (C) and 16h (D). The zero reaction control surface appeared identical to the 8h time point. All images depict a width of 2.7μm.

Figure 3 (B-D) shows that protein was retained in the tailored tubes following sequential washings with high salt, distilled water and detergent solutions. Indeed, numerous models of adsorption and immobilization have been established to rationalize protein binding [6]. While the dominant mode herein remains to be investigated, the results nonetheless support a strong association of protein on tailored polypropylene [7]. In contrast, native polypropylene (Fig. 3A) did not retain protein to any noticeable degree. Moreover, persulfate-treated or aminated polypropylenes with flat mesoscale topologies easily released their protein (unpublished data) in contrast to these findings reported for rough surfaces. Covalent linkers such as aldehyde groups [8] could retain protein on flat surfaces (unpublished data). The results implied that a mesoscale topology is particularly advantageous to realize high protein loadings, and crucial to achieve good retention in cases where protein-surface interactions are exclusively noncovalent.

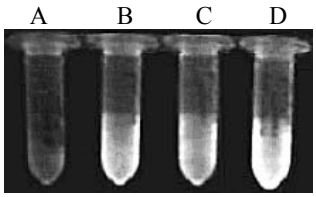

Fig. 3: Following successive washings, the fluorescence of dansyl-albumin had not decreased in surface-tailored tubes but was completely lost in the control tube. The tubes above depict the following functional moieties at the surface: A, native (methyl, methylene & methane); B, oxidized (carboxylic acid, ketone & hydroxyl); C, 3-aminopropylsilylated (amino and residual starting compounds); and D, 3-propanalylsilylated (aldehyde and residual starting compounds).

Clearly, these findings implied that the routine use of reaction-induced phase transformations could aid in the development of alternative mesoscale topologies. Such chemical approaches would complement methods based on lithography, self-organization & solvent casting.

Acknowledgements

This work was supported by Tübitak, Turkey, under project code MİSAG-194, and Setaş Kimya Inc., Turkey. A.T. thanks Mehmet Ali Gülgün of Sabancı University and İlhan Aksay of Princeton University for helpful suggestions. The SEM analyses were obtained at the Marmara Research Center, Turkey.

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