dimethylmethoxysilane. In preliminary experiments, this protocol had yielded the best results with respect to the surface roughness of the silanized alumina (as determined by AFM imaging), which should be as low as possible in order to not obstruct the pores. The surfaces were then reacted with fluorescein isothiocyanate, an amine-reactive fluorescein derivative. The success of this reaction was verified using a fluorescence microscope, by comparing surfaces with and without FITC treatment. The imprinted polymer was then synthesized by nanomolding on the nanoporous surface. For the fluorescein template, 4-VPy was found to be a suitable functional monomer in combination with MMA as filling monomer. The neutral filling monomer was added in order to keep the degree of crosslinking constant when the amount of VPy was varied. A porogenic solvent was not necessary in this imprinting recipe, as all binding sites are situated at the filaments' surface, and thus are easily accessible. After processing, the nanofilament-covered surfaces were incubated in a solution of fluorescein in DMSO to check for re-binding of the template. The amount of fluorescein adsorbed to the surface was determined by fluorescence microscopy followed by image analysis. The use of a laser scanning confocal microscope allowed for complete photobleaching of a small area on the surface, which provided a measure for the background signal. As shown in Figure 3A, an imprinted surface binds more fluorescein than a non-imprinted surface. In addition, the amount of bound fluorescein increases with the relative amount of functional monomer (4-VPy) in the polymer, which was not observed with the control polymer. A number of additional control experiments were performed to confirm the molecular imprinting effect and exclude surface-related artefacts. Polymers were cast on all surfaces at the different stages of preparation of the template surface. After incubation in a fluorescein solution, the bound fluorescein was quantified. Only the polymer that had been cast on an alumina surface with covalently attached fluorescein was able to bind fluorescein (Figure 3B). Binding of fluorescein to that polymer was inhibited in the presence of 9-hydroxyxanthene, a non-fluorescent sub-structure of fluorescein, which also confirms the presence of molecularly imprinted binding sites.

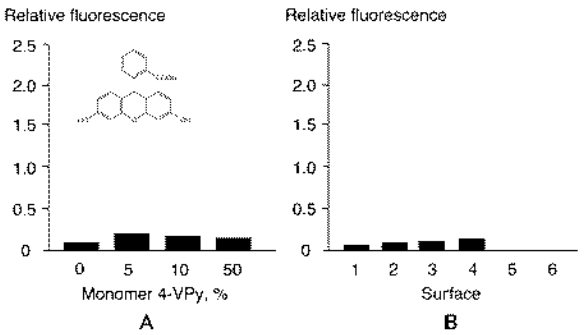

Figure 3 Relative fluorescence signals of nanostructured MIP surfaces after incubation with fluorescein. The values were calculated as : $F_R = (F - F_0)/F_0$, where F_R is the relative fluorescence intensity, F the fluorescence intensity of a given surface, and F_0 the signal obtained from a completely photobleached area at that surface. Light grey bars are MIPs, dark bars are non-imprinted controls. **A** : Polymers synthesized with different ratios of 4-VPy/(4-VPy+MMA); Insert : Structure of fluorescein. **B** : Signals obtained from polymers molded on different surfaces (1, aluminum; 2, alumina; 3, alumina incubated with FITC; 4, alumina silanized with aminopropyldimethylethoxysilane; 5, as 4, reacted with FITC; 6, as 5, but incubation with fluorescein during rebinding in the presence of 100 μM 9-hydroxyxanthene as competitor.

The wetting properties of nanostructured polymer surfaces were evaluated by contact angle measurements with water. A surface carrying nanofilaments of 600 nm length and 200 nm in diameter, synthesized from a rather hydrophobic monomer mixture containing divinylbenzene and methylmethacrylate showed a strong increase in contact angle compared to a chemically identical flat surface, from about 80° to 110°. On the other hand, if the monomer mixture was more hydrophilic (such as, a mixture of trimethylolpropane trimethacrylate and methacrylic acid), nanostructuration caused a decrease in contact angle. This shows that nanostructuration is able to enhance already existing surface properties.

Conclusions

In conclusion, we may say that we have used a combination of nanomolding and molecular imprinting to synthesize "all-in-one" polymer films with enhanced properties. We believe that these next-generation MIP films will be very useful in applications were specific molecular recognition in combination with large surface areas and good site accessibility are of importance. Nanostructuration is also able to alter the wetting properties of the surfaces. We are presently working on the synthesis of these surfaces in a more ordered way which will provide them with specific optical properties as a third integrated feature.

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Molecular Imprints in Nanostructured Polymer Surfaces - A New Generation of Biomimetic Materials for Chemical Sensors

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Introduction

The design and synthesis of biomimetic materials that are capable of binding target molecules with high specificity has been a longstanding goal in the analytical chemistry, biomedical and materials science fields. One of the most promising approaches towards producing such materials is molecular imprinting of synthetic polymers.[1-3] This technique involves using a molecular template, which directs the self-assembly of functional monomers that are subsequently co-polymerized in the presence of an excess of cross-linking monomers. The resulting molecularly imprinted polymers (MIPs) possess binding properties that often rival those of antibodies and enzymes, but with far greater stability than their natural counterparts.

Recently, there has been a growing interest in synthesizing MIPs in the form of thin films on surfaces, for example for use as recognition elements in chemical sensors. During initial experiments performed in our group on spin-coating of conventional photopolymerized MIPs, we have found that a potential problem during the synthesis of the films is their fast polymerization, which often prevents pore formation by the nucleation-based phase separation mechanism. Although we were able to address this problem by using a sacrificial polymeric porogen,[4,5] we felt that there was still much room for improvement. One alternative to porous films would be the use of nanostructured surfaces with a high aspect ratio. These can be produced for example using nanomolding on a template surface.

In the present work, we describe the preparation of nanostructured molecularly-imprinted surfaces using nanomolding on porous alumina. This produces surface-bound nanofilaments, which greatly increases the surface area of the material and should result in a faster mass transfer compared to porous films. Also of importance for the performance of thin MIP films is the actual localization of the imprinted sites in the material. Generally, these are evenly distributed throughout the bulk, however, for many applications it would be preferred to have the sites located at the surface of the material including the pore surface, as the sites will be better accessible and thus mass transfer will be faster, bulky labels can be used, etc. Several groups have addressed this problem with protocols for imprinting at interphases. For example, the imprint molecule can be immobilized onto a solid support such as, porous silica beads, prior to polymerization.[6,7] Thus, we have combined nanomolding with the immobilized-template approach to create polymer films that are structured both at the nanometric and molecular levels. The β-blocking drug propranolol and the dye fluorescein were used as model templates for imprinting.

Results and discussion

Glass surfaces with attached polymer nanofilaments were obtained based on a co-polymer of trimethylolpropane trimethacrylate (TRIM), 4-vinylpyridine (4-VPy) and methylmethacrylate (MMA) with the fluorescein template, or TRIM and methacrylic acid (MAA) with the propranolol template. Polymerization was initiated with UV light. The nanostructuration was obtained by nanomolding on a porous alumina surface. The pores in the alumina are obtained by electrooxydation with subsequent opening by acid treatment, and are all parallel to each other and perpendicular to the surface. Their depth and diameter can be fine-tuned, as described in the experimental section. Figure 1 shows representative scanning electron micrographs of TRIM-co-4-VPy-co-MMA polymer surfaces. The length and the thickness of the surface-bound nanofilaments can be adjusted within a certain range via the dimensions of the pores in the alumina layers. Diameters between 50 and 300 nm, and lengths between a few hundred nanometers and a few micrometers can easily be obtained. If one takes the surface in Figure 1c as an example, where the diameter of the filaments is about 200 nm with a length of 2 µm, the calculated surface area increases by a factor of 40 compared to a flat surface. In Figure 1d, the gain in surface area is even higher.

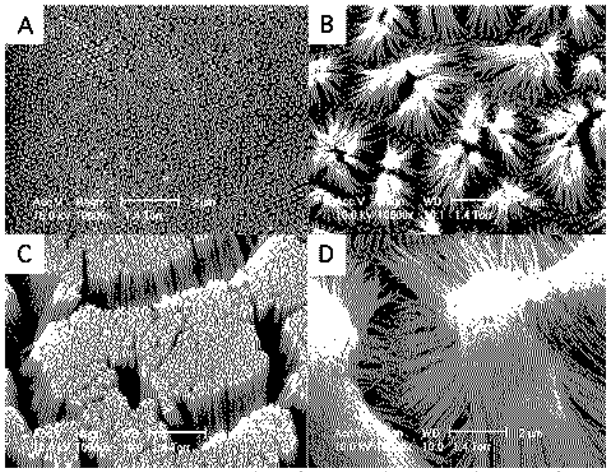

Figure 1 Scanning electron microscope images of non-imprinted polymer nanofilaments obtained with different experimental conditions (electrooxydation/phosphoric acid treatment durations) : short/long (A), short/short (B), long/long (C), long/short (D).

The next step was to synthesize these nanostructures in the form of molecularly imprinted polymers. Two different approaches were chosen, imprinting of the bulk filaments and imprinting of the filament surface. For bulk imprinting, the template was mixed with the monomers in a 1:8 ratio relative to MAA, and one volume equivalent of toluene was added to generate a gel-like, nanoporous structure of the filaments. This allows for access to the binding sites in the bulk of the filaments by diffusion. The nanostructured surfaces were then incubated in a solution of radiolabeled propranolol, and the amount of bound radioligand was determined using liquid scintillation counting. As can be seen in Figure 2, an imprinted surface bound a far higher amount of radioligand than a non-imprinted surface. When non-labeled R or S-propranolol was added to the incubation solution, the S enantiomer (the original imprinting template) had a concentration-dependent inhibitory effect on the binding of the radioligand, whereas the R enantiomer caused much less inhibition. These results clearly prove that the nanofilaments contain selective molecularly imprinted binding sites.

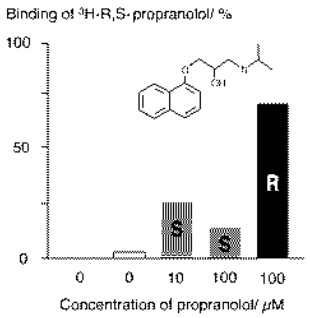

Figure 2 Binding of radiolabeled propranolol to nanostructured polymer surfaces in the presence of S or R propranolol as competitors (the letters in the bars indicate the competitor used). Filled bars : S-propranolol-imprinted polymer; empty bar : non-imprinted control polymer.

Despite the small diameter of the nanofilaments, which reduces diffusion distances and should greatly improve the access of the ligand to the binding sites, one might wish to even further reduce diffusion limits by creating binding sites only at the surface of the filaments. This is possible if the template is immobilized onto the alumina surface prior to imprinting, a technique that we have described for porous silica beads in an earlier publication.[6] To prove this concept with the nanofilaments, fluorescein was chosen as template molecule. The alumina surfaces were silanized with aminopropyl dimethylethoxysilane in order to introduce amino groups. This silanization was done in the gas phase using aminopropyl