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Investigation of a biofunctional polymeric coating deposited onto silicon microcantilevers

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Abstract

The paper deals with an appealing route to activate silicon microcantilevers (90, 110 and 130 μm long, 35 μm wide and 2 μm thick) for specific binding of biochemical species. The method consists in coating the underivatized microcantilevers with a biofunctional copolymer (based on *N*,*N*-dimethylacrylamide bearing silanating moieties) that was developed for low-density microarray assays on microscope glass slides. Coating deposition was obtained by dip-coating and its microstructure investigated by analyzing the resonance frequency values of bare and coated microcantilevers, by SEM and SFM imaging, SFM tip-scratch tests and XRR experiments. Results indicate that the coating is 2.5 nm thick and has a density of 1.22 g/cm^3. The coating surface is nanostructured, displaying nanoblobs, which are from few up to 20 nm wide and, on average, 1.6 nm high. The diameter of the biggest nanoblobs is of the same order of magnitude of the gyration radius of the copolymer chains, suggesting that nanoblobs may identify individual macromolecules.

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1. Introduction

Application of microcantilevers (MCs) to biosensing promises to be a breakthrough in biochemistry, life science and medicine [1–3]. Several experiments have been successfully performed in this direction, revealing DNA hybridization (including detection of single nucleotide polymorphisms, SNPs) [4–7] and detecting proteins and antibodies [8–11], single virus particles [12–14] or bacteria [15,16].

MC biosensor working principle is simple: the MC is functionalized with a probe that can selectively bind the target biochemical species. Adsorption and binding site interactions of the targets change the mechanical response of the MC system (because of the mass increase and/or the surface stress generated by changes in Gibbs free energy), providing the transduction/sensing mechanism.

Probes immobilization can be obtained through direct adsorption onto the MC surface [13,14,17] or through specific functionalization methods. Indeed, accessibility, stability and efficiency of surface functional groups are of crucial importance and may constitute decisive feasibility drawbacks for applications of MCs in biosensing experiments.

The use of thiolated molecules on gold coated MCs is the most common functionalization method for DNA [4–7], protein and antibodies [8–11]. Bacteria, viruses [18,19] and in some cases proteins [20] have been immobilized by activation techniques based on organosilanization. An alternative approach to gold coating and organosilanization chemistry is to coat the underivatized MCs with appropriate functional polymers. This approach has found wide application in MC chemical sensing [21–24]. On the contrary, to the best of our knowledge, it was adopted only once in MC biosensing by Gunter et al., who used a poly-ethylen-oxide (PEO) coating to immobilize vaccinia polyclonal antibodies [25].

In the present paper the possibility of activating silicon MCs by a co-polymeric thin coating based on *N*,*N*-dimethylacry-

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lamide bearing silanating moieties is assessed. This polymer was developed for DNA and protein microarray assays on microscope glass slides [26,27]. As showed in the previous paper [26], the deposition of a thin layer of this polymer represents a fast, inexpensive and robust method to covalently bind amino-modified DNA probe strands on silicon dioxide surfaces with high density. In addition, the film is stable in aqueous buffers containing various additives, even at water boiling temperature and, due to its hydrophilic nature and high homogeneity, it minimizes non-specific interactions. This feature is highly desirable for surface recognition bioassays, for which interaction specificity is often an issue. Finally, the macromolecular chains of the polymer act as spacer arms keeping the probe molecules away from the surface more effectively than self assembled monolayers (SAM) obtained by organosilanization.

The deposition of a thin film of polymer onto the underivatized MCs (90, 110 and 130 μm long, 35 μm wide and 2 μm thick) was obtained by dip coating according to the procedure previously devised for glass slides [26]. It is worthwhile to notice that, because of the MC size, detailed insight into the coating microstructure resulted a challenging task. This goal was achieved by integrating the analysis of the variation of the resonance frequency of the coated MCs with respect to the bare MCs with the pieces of information obtained from the images of scanning electron and scanning force microscopies (SEM and SFM), SFM tip-scratch tests and from X-ray reflectivity (XRR) data.

2. Experimental

The ter-polymer (DMA-co-NAS-co-MAPS) contains *N*,*N*-dimethylacrylamide (DMA), *N*-acryloyloxysuccinimide (NAS) and 3-(trimethoxysilyl)propyl methacrylate (MAPS), respectively at 97%, 2% and 1% total monomer moles. The procedures used to prepare and characterize the polymer are reported in Ref. [26]. Hereafter it will be referred as (DMA-NAS-MAPS).

We used commercial silicon MC arrays made of three MCs (NSC12/Tipless/No Al by MikroMasch, Tallinn, Estonia). Each array consists of three MCs of different lengths, namely 90, 110 and 130 μm, and of the same width and thickness: 35 and 2 μm, respectively. Resonance frequency measurements were performed in air, at room temperature using a scanning probe microscope (SPM) head with an integrated laser lever readout (Jeol, Tokyo, Japan). The Jeol SPM apparatus, equipped with silicon tips by NT-MDT, Russia, was also used for SFM experiments. Scanning electron microscopy (SEM) was performed with a EVO 40 Microscope by LEO.

Polymer coating experiments were organized in two separate batches. The coating procedure consisted of three steps. Firstly the arrays were washed with acetone (Carlo Erba, Milan, Italy) and after drying, they were immersed for 30 min in a solution of the polymer (1% w/v in a water solution of ammonium sulphate at 20% saturation level). Finally, they were accurately washed with demineralized water and dried under vacuum at 80 °C for 30 min.

The polymer deposition procedure previously assessed [26,27] requires a pretreatment with HCl 1 M and NaOH 1 M. Such pretreatment is intended to activate the surface silanols (a thin layer of native silicon dioxide is present on the silicon surfaces of the MCs), maximizing the number of hydrogen bonds with the polymer carbonyl groups and ensuring the physisorption of a higher quantity of polymer. However, the alkaline pretreatment resulted too aggressive and changed the mechanical properties of MCs. This was witnessed by an increase of the MC resonance frequency in the order of tenth of kHz. In view of this, the MCs were just carefully washed with acetone to eliminate superficial contaminations.

In order to analyze the polymeric layer with X-ray reflectivity (XRR), a silicon wafer slide (approximately 1.5 cm large and 2.5 cm long) was coated following the same procedure and using the same polymer solution of the second MCs batch. This allowed to obtain the best matching between the MCs and the wafer coatings, as it was verified by SFM analysis. XRR data were collected with a Bruker D8 Advance reflectometer equipped with a Göbel mirror. The X-ray beam (Cu Kα radiation, $\lambda = 0.154$ nm) was collimated by proper slits in order to set the cross section to 600 μm × 1.5 cm. All XRR profiles were analyzed with the REFSIM software [28] in order to extract information on the layer density, thickness and roughness [29].

3. Results and discussion

The DMA-NAS-MAPS coating on the MCs was imaged by SEM (Fig. 1) and quantitatively investigated by analyzing the negative shift of the MC resonance frequency due to the mass of the adsorbed DMA-NAS-MAPS layer (Fig. 2).

The SEM images of a DMA-NAS-MAPS coated MC collected by secondary electrons and backscattered electrons are reported in Fig. 1a and b, respectively. The secondary electron image, which probes the surface morphology, evidences that the polymeric layer is continuous, presenting micron size corrugations localized far from the MC base. These features disappear in the backscattered electron image. Since backscattered electrons mainly probe the surface chemical composition, this result indicates that the mentioned features are due to inhomogeneities of the polymeric layer.

In order to evaluate the deposited mass of DMA-NAS-MAPS, we used the general equation for the resonance frequency (f) of an oscillating cantilever in presence of fluid damping and added mass [18,30]:

$$f = 0.325\sqrt{\frac{K}{m + m_f + \Delta m}}, \quad (1)$$

where K is the cantilever spring constant, m the cantilever mass, m_f the mass of the fluid film (mainly coming from ambient moisture) adherent to the cantilever surface during oscillation and Δm is the added mass. The constant 0.325 is a form factor that accounts for the cantilever geometry. Eq. (1) is derived