Sol-gel Silane Films for DNA Microarray Experiments

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ABSTRACT

Preparation and some characteristic properties of sol-gel derived APTMS-TMOS (APTMS, $H_2NCH_2CH_2CH_2$-$Si(OCH_3)_3$; TMOS, $Si(OCH_3)_4$) hybrid films are discussed on the basis of experimental AFM, FTIR and wettability measurements. The AFM measurements reveal uniform surface of the films that consist of densely packed polysiloxane particles. The films are stable in aqueous environment up to certain relative content of APTMS that extends their applicability in various technologies. As an example, the films were tested as substrates for immobilization of 25-mer oligonucleotide DNA, and results indicated several advantages compared to commercial aminosilanized slides.

Keywords: sol-gel hybrid films, uniform surface, atomic force microscopy, DNA immobilization

1 INTRODUCTION

Silanization of hydroxyl-terminated substrates is an effective and frequently used procedure for modification of chemical and physical properties of the substrates as well as to covalently immobilize a variety of compounds onto them. Therefore, silane coatings serve a number of applications such as protective coatings or adhesion promoters on metal surfaces [e.g. 1,2], adhesives in industrial paints [e.g. 3], selectively binding surfaces for tethering biological molecules in biosensor and DNA chip design [4,5], in scanning probe microscopy (SPM) studies of biomolecules [6], and in chemical force microscopy as probe functionalizing agents [7]. Recently, self-assembling of silane monolayers has received growing attention because it can lead to new technological applications. Focus has mainly been on formation of uniform monolayers of long-chained organosilanes, where alkyltrichlorosilanes, particularly octadecyltrichlorosilane (OTS, CH_3-$(CH_2)_{17}$-$SiCl_3$) on different hydroxylated surfaces such as oxidized silicon or mica [8,9] are among the most studied systems. In contrast, alkyltrialkoxysilanes bearing short tail group have been studied only in a limited number of cases [e.g. 10].

Since introduction [6] as a reliable route for immobilization of DNA for SPM studies the silanization of mica or glass using trialkoxyaminopropylsilanes, particularly 3-aminopropyltriethoxysilane (APTES, $H_2NCH_2CH_2CH_2$-$Si(OC_2H_5)_3$) has become a common procedure in similar research [11,12,13]. However, the formation of uniform trialkoxy- and trichlorosilane monolayers is impeded by self-polymerisation of silane, caused by the trace quantities of water in reaction medium [14]. Considering this, it has been claimed that the surface of APTES layer is heterogeneous and varies from sample to sample [15], and it is not stable in aqueous medium [12].

In our recent work we proposed an alternative silanization technique that substantially improved homogeneity and smoothness of the surfaces [16]. Mica substrate was dip coated with partially pre-polymerized APTMS sol that was thereafter gelled in humid air. Still, instability of the films in water that can be explained with low-rate cross-linkage between individual siloxane molecules because of steric hindrance of aminopropyl groups, needed to be improved. In this work we present our further developments of the approach. APTMS was co-polymerized with TMOS in order to favour gelation of the precursor material, and subsequently gelled on glass substrate. Essential properties of the films were characterized by IR spectroscopy, MALDI TOF mass spectrometry, wettability, and atomic force microscopy (AFM) measurements. The potential of the films as substrates for immobilization of 25-mer oligonucleotide DNA was tested and their advantages compared to commercial analogues (SAL-1 slides, Asper Biotech Ltd., ref. 17) are discussed.

2 EXPERIMENTAL PROCEDURES

In brief, a series of samples were prepared varying molar concentration of APTMS relative to TMOS for characterizing dependence of surface topography on the ratio of silanes using AFM imaging. Thereafter, wettability of the surfaces of the same series was measured and characterized by determining contact angles of the surfaces to correlate relative concentrations of APTMS/TMOS and hydrophobicity of the surfaces. Presence of several

chemical groups was verified by IR spectroscopic analysis. One of the possible applications of the new method, DNA spotting was tested by a standard DNA spotting method using 25-mer oligonucleotide DNA (1 part of Cy3 3'labelled 5'-aminomodified 25-mer oligonucleotide DNA mixed with 100 parts of unlabelled 25-mer oligonucleotide DNA).

3 RESULTS

3.1 Spectroscopic measurements

For FTIR measurements, freshly prepared KBr pellets were coated with solutions of pre-polymerized precursor of APTMS-TMOS in methanol. The absorptions corresponding to NH_2, SiOH, SiOSi and CH_2 vibrations were confirmed.

MALDI TOF MS measurements were performed using 1,8,9-trihdroxyanthracene (dithranol) and 2,5-dihydroxy benzoic acid (DHB) as matrixes. The hybrid material had molar masses in range of 800 – 1500 amu. As expected, the spectra had very complicated structure that contained different "families" of oligomers and therefore was not very informative. It was estimated that such mass distribution corresponds to the oligomers containing approximately 7-12 monomers.

3.2 The surface of APTMS-TMOS films

APTMS-TMOS 0:1 (0:1 is relative molar concentration) film exhibited a uniform and smooth surface (average vertical difference 5 nm per 1 μm scan). APTMS-TMOS 1:10 film showed surface consisting of grains with several to a hundred nanometers in diameter and average height distribution of 20 nm/μm (Fig. 1a). The surfaces of APTMS/TMOS 1:5 and 1:3 films were similar to 1:10 film. Starting from APTMS-TMOS 1:1 film the surface profiles ranged between two nanometers, thus showing practically featureless topography in micrometer scale (Fig. 1b).

3.3 Wettability of APTMS-TMOS films

The contact angle measurements indicated that APTMS-TMOS 0:1 and 1:10 films were completely wettable, e.g. no water drops formed on their surfaces. The contact angle of APTMS-TMOS 1:5 film was 14 degrees and abrupt jump to 40 and further on to 60 degrees was observed in the case of APTMS-TMOS 1:3 and 1:1 film, respectively. The contact angles of APTMS-TMOS 3:1, 5:1 and 10:1 films also remained in proximity of 60 degrees, whereas in the case of APTMS-TMOS 1:0 film the contact angle dropped to 50 degrees.

3.4 DNA immobilised to APTMS-TMOS films

Fig. 2 illustrates the binding efficiency of DNA 25-mers to silanized slides. In the case of APTMS-TMOS 0:1 film no binding was detected, which is because of the absence of functional groups in the film. The binding to APTMS-TMOS 1:10 and 1:5 films was in the order of 10% of the SAL film, indicating to the presence of functional groups on the slide. Further increase in the ratio of APTMS

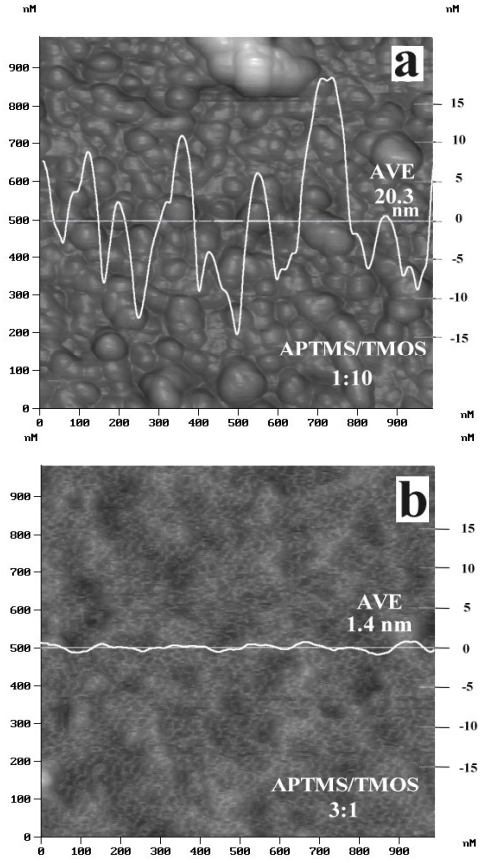

Fig. 1. Semi-contact mode AFM images of APTMS-TMOS hybrid films; scan range 1x1 μm^2. The scale bar on the right side of the images corresponds to the line profiles drawn in the middle of the scan.

(APTMS-TMOS 1:3 film) gave considerable rise in the amount of DNA immobilised on the surface, but the signal still remained 50% below the level of SAL-glass. The SAL-glass signal was exceeded in the cases of APTMS-TMOS 1:1 and 3:1 films showing substantially higher binding efficiencies (140 % and 135 % of SAL signal, respectively). Further increase in the relative amount of APTMS in the film led to scattered DNA spots and thus, no comparative binding efficiencies could be obtained. The scattering can be explained by dissolution of the films in aqueous environment due to lower rate of cross-linking between aminosilaxane oligomers.

The dimensions of the DNA spots decreased with the increase of the amount of APTMS in the films (Fig. 3), which can be explained by the decrease in wettability, caused by additional amount of hydrophobic aminopropyl groups. The dimensions of the spots on APTMS-TMOS 1:3, 1:1 and 3:1 films were close to the spot sizes on SAL-glass. Starting from APTMS-TMOS 5:1 film, the spots

were not clearly outlined due to the dissolving of silane coating in aqueous medium.

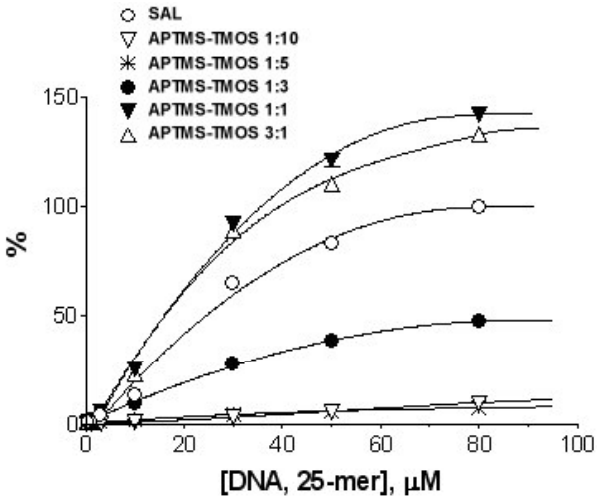

Fig.2. The binding curves of 25-mer oligonucleotide DNA to APTMS-TMOS hybrid films, normalized to the signal of 80 µM DNA spot on the SAL-slide. Each data point corresponds to an average fluorescence intensity of 16 independent spots.

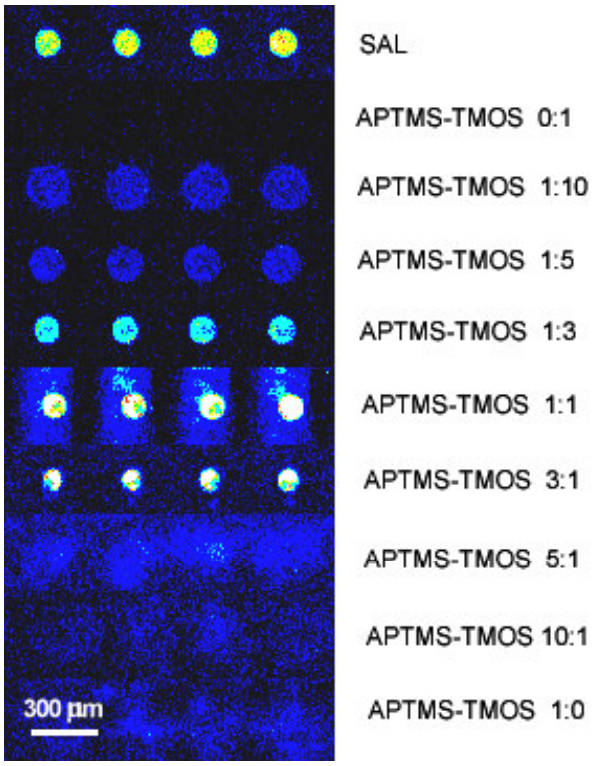

Fig.3. Fluorescence images of 80 µM DNA spots on SAL-slide and on APTMS-TMOS hybrid films.

CONCLUSIONS

It was shown that APTMS-TMOS hybrid films have potential as substrates for immobilisation of biomolecules. The ratio of APTMS/TMOS determines the density of functional groups on the surface. The degree of polymerization can be readily monitored using FTIR spectroscopy and MALDI TOF MS methods, respectively. Such films are stable in aqueous environment up to APTMS content 3:1 in APTMS-TMOS hybrid film. The binding rates of DNA 25-mers to APTMS-TMOS 1:1 and 3:1 films were ≈ 140 % of the binding to commercial SAL-glass, which we believe is due to the uniform distribution of functional groups. Furthermore, the use of APTMS-TMOS hybrid films increases reproducibility of the fraction of immobilised biomolecules, because of the formation of new surface that is virtually independent of the underlying topography of the glass support. Still, the optimisation of the preparation of precursor material and films, as well as the detailed binding characteristics of biomolecules remain to be resolved.

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