

Homogeneous and smooth siloxane films for AFM visualization of immobilized biomolecules

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Abstract

A preparation technique of siloxane films based on gelation of linearly polymerized tetraethoxysilane ($\text{Si}(\text{OC}_2\text{H}_5)_4$, TES) and 3-aminopropyltrimethoxysilane ($((\text{OCH}_3)_3\text{SiCH}_2\text{CH}_2\text{CH}_2\text{NH}_2$, APTMS) was developed. These films were characterized using AFM, FTIR and NMR methods.

Introduction

One of the common procedures for atomic force microscopy (AFM) studies of biological macromolecules uses amino-terminated alkoxy silanes as cross-linking agents between hydroxylated surfaces and biological species. A simple protocol for covalent binding of DNA and RNA to 3-aminopropyltriethoxysilane (APTES) -treated mica was introduced a decade ago by Lyubchenko *et al* [1]. In their work they described the preparation of siloxane surfaces by treating mica substrates with dilute solutions of APTES (0.1 μM – 0.1 M) in toluene as well as by chemical vapor deposition (CVD) of APTES to mica. The properties of siloxane surfaces prepared by similar manner have been subject for discussion since their introduction as they constitute an important and widely used class of supports for biomolecule immobilization. As a potential problem that somewhat hinders their application as supports for AFM-visualization many groups have pointed to poor structural reproducibility of silanized surfaces [1-5]. The formation, structure and thickness of siloxane layer in liquid environment have been found to be extremely sensitive to the trace quantity of water in solvent and on the support, leading to self-polymerization of silane and formation of three-dimensional nanosized siloxane clusters [5]. Moreover, the properties of siloxane film depend on the concentration of silanizing agent in solution [1,4,5] and on the presence and density of silanol groups (Si-OH) on the support [6]. Similarly, CVD-grown films of alkoxy silanes exhibit high sensitivity to moisture during formation and concentration of silane vapor [7], though this process can be controlled more easily than liquid phase deposition [2,7]. However, there remain still a number of unresolved issues that retard wide spread application of CDV silanization.

Silanization of hydroxylated surfaces with alkoxy silanes in the presence of water is a topic of sol-gel chemistry that has been intensively studied already in 1980's [8-10]. In this work we present new results on the preparation of reproducibly smooth siloxane films by gelation of linearly polymerized TES or APTMS solution in methanol. We show the possibility to obtain homogeneously smooth siloxane surfaces, which do not contain irregularities, grains, clusters and domains of different dimensions. We emphasize that siloxane surfaces prepared by sol-gel method are homogeneously smooth over unlimited range of area, which make them excellent supports for AFM studies of immobilized biological species.

Experimental

Detailed protocols for linear polymerization of TES and APTMS (both Sigma Chemical Co) have been described previously [11]. Briefly, one part of TES or APTMS was mixed with two parts of water and heated up close to boiling point of silane in open air under constant stirring. The reaction was terminated by introducing cold dry methanol to the highly viscous mixture, which was thereafter dried by azeotropic distillation with heptane. The highly viscous residue was dissolved in dry methanol, sealed, and stored at room temperature. A series of samples was prepared for AFM-imaging by dropping different concentrations of this solution onto freshly cleaved mica surface. Prior to imaging, the samples were subsequently hydrolyzed in humid air for 12h at room temperature and baked at 90 °C for 12h. AFM measurements were performed with *SMENA-B* (NT-MDT, Russia) microscope working in semi-contact (tapping) mode in air using Ultrasharp Noncontact “Golden” silicon cantilevers (NT-MDT, Russia).

The chemical composition of linearly polymerized silanes was determined using an Interspec 2020 FTIR spectrometer (Interspectrum, Estonia) and a Bruker AC200P (Spectrospin, Switzerland) NMR spectrometer (for details see [11]).

Results and discussion

AFM images of linearly polymerized TES and APTMS films demonstrate highly homogeneous surface showing height difference of about 1 nm over $1 \times 1 \mu\text{m}^2$ area (Fig. 1). Several measurements on different places on $1 \times 1 \text{cm}^2$ sample area confirm this practically featureless topography to be characteristic for siloxane films prepared from linearly polymerized TES and APTMS. No qualitative difference was discovered between topographies of TES and APTMS films.

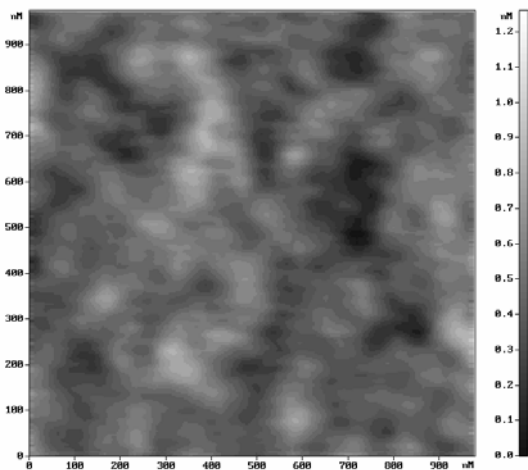


Fig. 1. $1 \times 1 \mu\text{m}^2$ tapping mode AFM image of linearly polymerized TES film.

It has been shown by different groups that in the cases of most alkoxides intensive stirring of mixture of water/alkoxide at molar ratio 1:1 to 4:1 in opened systems generates preferentially linearly polymerized siloxane chains [8,10,12,13]. This so-called linear polymerization is most profoundly described on the basis of TES, which we have chosen as reference system for APTMS. TES has no functional group for direct covalent linkage of biomolecules, however, its polymerization can be generalized to other alkoxides such as APTMS.

High-resolution AFM phase imaging of linearly polymerized TES film clearly shows individual molecules of linear polymeric particles (Fig. 2). The result is in good agreement with alternative experimental data that predicted linear formation of these molecules [8] but so far to the best of our knowledge no visual evidence of this fact has been presented.

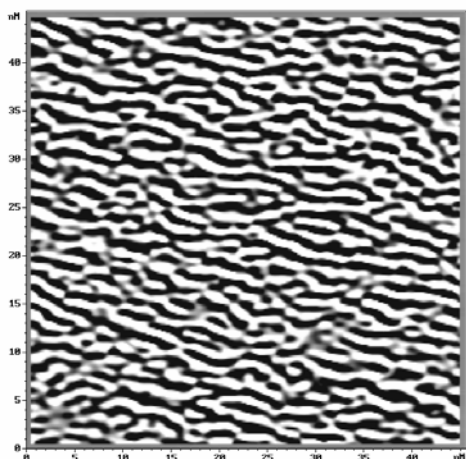


Fig. 2. 50x50 μm^2 phase image of linearly polymerized TES film.

NMR and FTIR spectra of linearly polymerized APTMS confirm the presence of aminopropyl chain, as three nonequivalent aminopropyl groups giving three signals in 9 ppm region of ^{13}C NMR spectra can be distinguished. The small sharp signals in ^1H NMR spectra at 3.17 ppm and in ^{13}C spectra at 48.49 ppm indicate the presence of OCH_3 group. The 7.3 % content (normalised to the aminopropyl chain) of methoxy group offers a good possibility for linking linearly polymerized APTMS to SiOH group of solid surfaces. Prepared linearly polymerized APTMS also contained 22% of SiOH group, needed for cross-linking of individual linear molecules to form three-dimensional amorphous siloxane film.

Conclusions

Application of sol-gel method in silanization procedure of surfaces is successful and promising technology solving several problems that limit usage of other existing methods. Surfaces created by the new method possess much higher degree smoothness than currently available without sacrificing other important characteristics of the surface e.g. presence of active functional groups. Therefore their application in AFM visualization of biological samples improves significantly quality of the acquirable data as well as widens range of potential research samples. We have shown a specific procedure for applying the sol-gel method in treating mica surface using linearly polymerized TES and APTMS. Either of them can be used for making smooth siloxane surfaces. It should be stressed that the uniform and homogeneous surface extends over very wide area (in the order of square centimeters) making the method especially valuable for many practical applications.

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