Functionalization of AFM tips by atmospheric pressure plasma polymerization

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Abstract: Atmospheric pressure plasma polymerization represents a new technological solution to coat various substrates with functional polymer layers. We employed this technique to modify the surface properties of standard atomic force microscope (AFM) tips. Using a dielectric barrier discharge reactor we synthesize thin polymers films of styrene. The operational parameters of our polymerization process were identified and the dependence of deposition rate on Yasuda’s parameter is presented. Data on chemical structure of our plasma polymers are obtained from FTIR, XPS, NMR spectroscopy and gel permeation chromatography (GPC). The functionalized AFM tips were used to measure the adhesion force between the bovine serum albumin and polyethylene terephthalate (PET) surfaces with atomic force spectroscopy mode.

Keywords: plasma polymerization, AFM tips, biological molecules

1. Introduction

Atomic force spectroscopy is a versatile tool for quantitative characterization of surface properties: morphology images, roughness, phase images, adhesion force measurement. Involving the use of the Atomic Force Microscope (AFM), force spectroscopy is a dynamic mode of operation which allows the study of mechanical properties of biological molecules and chemical bonds. Force spectroscopy measures stretching or torsional forces between the tip and a molecular layer.

Molecular force spectroscopy study using the AFM has become an established technique and helped to reveal important characteristics of many biological molecules such as DNA molecules, enzymes, plasma proteins and other molecular complexes or structures of biological interest [1].

In This study was focused on the determination of the optimum parameters of the polymerization process in the dielectric barrier discharge (DBD) at atmospheric pressure and then, this method was used for AFM tips functionalization. Measurements of the adhesion force between the bovine serum albumin (BSA) immobilized on the tip and polyethylene terephthalate (PET) surfaces were taken using force spectroscopy mode of AFM.

2. Experimental methods

AFM force spectroscopy is based on the following principle: the cantilever’s tip is attached to one end of the biomolecule to be analyzed, the other end being held by a substrate. The cantilever is then moved using a piezocontroller to exert a tensile load on the biomolecule. Using this mode of operation of the AFM, characteristic force curves for the biomolecule are be obtained [2].

The force - distance curves was recorded with a silicon nitride tips (MikroMash CSC12) with typical spring constant $k = 0.03$ N/m in PBS solution. The functionalization of the tips was obtained after a polymerization reaction induced in a dielectric barrier discharge (DBD) at atmospheric pressure (Fig 1 a). The AFM tips were placed on the ground electrode and the monomer was styrene. The
helium gas flow was 3 l/min for the working gas and 0.5 l/min for monomer bubbling. Positive high voltage monopolar pulses were delivered using pulse amplifier (Trek Inc) connected with a function generator (Tabor Electronics Inc). The discharge parameters were: high voltage amplitude, $U = 2kV$, repetition rate, $\nu = 2kHz$ and duration of polymerization, 5 min.

Figure 1. Experimental set-up (a), typical signals of the applied voltage and discharge current (b)

Figure 1 (b) presents the time dependence of the applied voltage and the discharge current. The discharge current consists in two symmetrical peaks during each applied voltage pulse with a peak value around 20 mA; well defined shapes are an indication for the diffuse mode of DBD.

After the coating with the plasma polymer, the AFM tips were immersed in 1 mg/ml BSA solution for 20 min and the force-distance curves was recorded immediately after the AFM tip functionalization.

3. Results

The electrical parameters were carefully chosen in order to obtain plasma polymerization reactions with high deposition rate and high concentration of active species [3,4]. Thus, the dependence of polystyrene deposition rate was analyzed as function of a control parameter, W/FM, in order to identify the domains of plasma polymerization reactions (figure 2). For low values of W/FM, the deposition rate is increasing up to 3.8 nm/s delimitating the monomer sufficient region. The competition zone is identified at the maximum deposition rate which corresponds to 3200 MJ/Kg. Here we suppose that is retrieved the maximum efficiency of the plasma polymerization system. As W/FM increases, the deposition rate is linearly decreasing, delimitating the monomer deficient region.

Figure 2. Domains of plasma polymerization reactions of styrene

Figure 3 shows comparative FT-IR spectra of plasma polystyrene in 3800 – 600 cm$^{-1}$ spectral region. Plasma polystyrene spectrum contains absorbance bands of aromatic C-H stretching at
3661 cm$^{-1}$ and 3026 cm$^{-1}$; methylene C-H stretching at 2931 cm$^{-1}$; methyl (CH3) C-H stretching at 2968 cm$^{-1}$; aromatic C=C stretching at 1685, 1600 and 1493 cm$^{-1}$; methyl C-H bending at 1450 cm$^{-1}$; and C-H aromatic groups from benzene ring in the range 1073 – 700 cm$^{-1}$.

![Figure 3. Typical FTIR spectrum of plasma polystyrene films](image3.png)

Figure 3. Typical FTIR spectrum of plasma polystyrene films

The structure of the plasma polymerized styrene was determined by Nuclear Magnetic Resonance (NMR) spectroscopy. As our NMR apparatus analyses liquid solutions, the plasma polystyrene thin films were dissolved in dimethyl sulfoxide. The NMR spectrum for polystyrene contains a major peak at 7.22 ppm which reveals the presence of benzene ring in the plasma deposits. Moreover, we found minor peaks at 0.856 ppm and 1.236 ppm which are assignable to n-hexane type groups. Thus, we assume that the plasma polystyrene films contain intact benzene rings but also, small percentage of benzene ring is broken into linear type compounds.

XPS technique was utilized as complementary method for FT-IR and NMR measurements. The XPS spectrum corresponding to plasma polystyrene films reveals the presence of carbon (C 1s peak at 284.6 eV) and oxygen (O 1s peak at 531.6 eV). The chemical composition is 90% C 1s and 10% O1s. The C1s envelope can be deconvoluted in four distinctive peaks assignable to C-C, C=C and C-H bonds (284.6 eV), C-O bonds (286.1 eV), C=O bonds (287.6 eV) and the $\pi-\pi^*$ shake-up satellite (291.4 eV). The presence of $\pi-\pi^*$ shake-up satellite confirms the existence of intact benzene rings in the polymer structure, which was indicated also by the NMR results. More, the presence of C-O and C=O bonds in the XPS spectrum reveals that the plasma polystyrene films are partially oxidized; the results are also in a good correlation with the infrared spectrum.

![Figure 4. XPS spectrum of the deconvoluted C 1s peak corresponding to plasma polystyrene films](image4.png)

Figure 4. XPS spectrum of the deconvoluted C 1s peak corresponding to plasma polystyrene films

Using gel permeation chromatography (GPC), the molecular weight of plasma polystyrene films was measured. Thin solid films, initially deposited onto the glass substrates, were dissolved in dimethyl formamide. The molecular weight of plasma polystyrene is around 2724 amu.

![Figure 5. SEM image of the plasma polystyrene coated AFM tip after the immersion in the BSA solution](image5.png)

Figure 5. SEM image of the plasma polystyrene coated AFM tip after the immersion in the BSA solution
The bovine serum albumin was identified on AFM tips by X-ray Photoelectron Spectroscopy (XPS) and in SEM images (Figure 5). In the XPS spectra we analyzed the specific peaks of silicon, carbon and oxygen. After the plasma polymerization, the tip is covered a polystyrene layer, identified from the specific bonds of carbon and oxygen. Immobilization of BSA onto the tip was proved by the presence of the N1s peak, corresponding to the amidic groups of BSA chain at 399 eV.

Figure 6. Measurements of the cantilever deflection as function as distance for the coated AFM tip with BSA

The deflection - distance curves, after the AFM tips functionalization, show rupture events that involve molecule – substrate broken bonds. The adhesion force was calculated using the Hooke's law:

\[ F_{ad} = -k \Delta x \]

where \( k \) is the spring constant of the cantilever, and \( \Delta x \) is the movement of the piezocontroller in nm. The value of the adhesion force between the BSA molecules and the PET substrate are equal to few hundreds piconewtons. Thus, for a single BSA molecule localized on the tip the adhesion force between the substrate and this molecule can be evaluated to 760 pN [5].

4. Conclusions

The AFM working in the force spectroscopy mode is one of versatile tools helping to reveal important characteristics of many biological molecules such as DNA, enzymes, drugs and other molecular complexes or structures of high biological activity. In force spectroscopy the most important issue is the preparation and functionalization of the AFM tips. For this reason we developed a method to functionalize the AFM tips based on the polymerization reactions induced by a dielectric barrier discharge (DBD) working at atmospheric pressure. Plasma functionalization allows a good attachment of the BSA molecules onto the AFM tips and subsequent evaluation of the adhesion forces between these biological molecules and a polymer substrate.

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References