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# Structural–mechanical and antibacterial properties of a soft elastic polyurethane surface after plasma immersion $N_2^+$ implantation

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ABSTRACT

The surface of elastic polyurethane treated by plasma immersion $N_2^+$ ion implantation at different fluences has been investigated. A folded surface structure is observed in all cases. Analysis has been performed to study the structural (roughness, steepness and fraction of folds, fractal characteristics), mechanical (stiffness, adhesion force between the AFM probe and the material) and wetting properties of surfaces. Under uniaxial stretching the cracks orthogonal to the axis of deformation and longitudinal folds are formed on the examined surfaces. After unloading the initial structure of the surface of deformed materials exposed to low fluences becomes smoother and does not recover, i.e. it has plastic properties. By contrast, the structure of the surfaces of materials subjected to high-fluence treatment recovers without visible changes and the cracks are fully closed. The study of *Staphylococcus* colonies grown on these materials has demonstrated significant reduction (from 3 to 5 times) in the vitality of bacteria on treated surfaces. This result was repeated on samples after 11 months of storage. Such antibacterial properties are primarily related to the structural changes of the surfaces accompanied by the increased hydrophilicity.



## 1. Introduction

Metals, ceramics and polymers are widely used in modern medicine materials. One of the commonly used polymers for the manufacture of biomedical devices is polyurethane (PU). Depending on the composition and preparation techniques the mechanical properties of the PU can vary over a wide range from soft elastomers to stiff plastics. PU is suitable for creating catheters, cardioimplants, breast implants, interphalangeal prosthesis, etc.

Currently, there are plenty of studies on surface modification of materials. These studies are intended to improve the quality of biomedical products (anti-bacterial properties, biocompatibility with living tissues). Surface modification can be done by different methods: mechanical and chemical techniques, plasma treatment, plasma immersion ion implantation, carbon sputtering [1–6], etc.

The plasma immersion ion implantation (PIII) has some advantages. Modified layer is formed in the material itself, and there is no distinct boundary between the modified surface layer and the bulk material. Plasma treatment modifies the chemical structure and hydrophilicity of the surface, and this significantly influences protein adsorption [7]. Wang et al. [8] revealed that the PIII treatment of PU in acetylene medium affected platelet adhesion. Bax et al. [9] studied the influence of the PIII treatment of the PU surface on the adhesion behavior of tropoelastin — protein responsible for the adhesion and growth of tissue cells. It has been found that application of silver [10] or copper [11] ions improves the antimicrobial properties of the material.

The influence of PIII on the structural–mechanical properties of the surface of PU is still poorly understood. Besides, in most works a relatively stiff PU (e.g., [9] — 35 MPa) is used. The PIII of soft elastic PU is a promising technique, which can also be used to improve the quality of movable, flexible medical devices. The mechanical compatibility between the material and the coating is important for the longevity of materials. It must satisfy operational conditions and loads. That is, the modified polymer layer must be strong enough to avoid delamination in the loading mode.

The present work focuses on the structural and mechanical characteristics of the surface of elastic polyurethane subjected to the PIII treatment at different ion beam fluences. The effect of the treated surfaces on the adhesion of colonies of *Staphylococcus epidermidis* was examined.

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The atomic force microscopy (AFM) in nanomechanical mapping regime was used to study the structure of the surface and its local mechanical properties. The formation of fractal folds was observed on the treated surfaces.

With increasing treatment time the surface structure becomes more significantly ramified, its stiffness increases and an adhesion force between the AFM probe and the material is reduced. The properties of surfaces were studied in the deformed (stretched) and undeformed (unstretched) states and after dynamic loading. Depending on the fluence and consequently on the structural–mechanical properties of the surface, a 5-fold reduction in the viability of bacterial films formed on the surface of the materials was achieved.

## 2. Materials and methods

### 2.1. Preparation of polyurethane composition

A polyurethane composition used in this study is a two-component system of a prepolymer (simple oligoether, mol. wt. 2300, based on polypropylene oxide and 2.4-toluene diisocyanate) and a hardener (aromatic diamine dissolved in the polyol) in a weight ratio of 100:46.2.

The estimated amount of prepolymer was heated to 50 °C and vacuumed for 7 min. Then it was mixed with a hardener mixture heated to 60 °C. The mixture was stirred and then vacuumed again at 50 °C for 7 min. The vacuumed mixture was poured into a mold and solidified in a heat chamber at 100 °C for 20 h. Then the material was cooled at room temperature. The average thickness of polyurethane plates was 1.5 mm. The mechanical characteristics of the plates are stabilized in 1–2 weeks.

### 2.2. Surface treatment

The samples were subjected to a plasma immersion ion implantation of nitrogen ions $N_2^+$. A source of electrons with plasma cathode based on a glow discharge was used to generate plasma in the vacuum chamber. The chamber was filled with nitrogen at a rate of 50 ml/min, and the pressure of a working gas was set to 0.35 Pa. A beam of low-energy electrons (up to 50 eV) with a diameter of 80 mm and a current of 0.5–0.6 A was formed in the double layer of space charge near the grid of plasma cathode. An electrically isolated holder of samples was located inside the vacuum chamber at a distance of 100 mm from the grid of electron emitting source. The temperature of the sample is measured using a thermocouple located on the holder. The sample was placed in the holder and closed by metal mesh with a cell size 0.6 × 0.6 mm. Pulsed DC (50 kHz) negative bias voltage with amplitude of 1 kV was applied to the holder. The plasma ions are generated by the electron beam and accelerated in the double layer of space charge region, which was created near the mesh. The average ion current density was 15 μA/cm$^2$. The fluence $F$ determined by the time of treatment $t$ was: a) $2 \cdot 10^{15}$ ions/cm$^2$ ($t = 20$ s); b) $2 \cdot 10^{16}$ ions/cm$^2$ ($t = 204$ s); and c) $2 \cdot 10^{17}$ ions/cm$^2$ ($t = 2040$ s). The temperature of the first two samples did not exceed 80 °C, the last — 130 °C. These temperatures measured at the sample holder were less than the temperature of thermal degradation of PU — 170 °C.

Analysis of the estimated distribution of the penetration depth of nitrogen ions (1 kV) into PU (density — 1.24 g/cm$^3$, the elemental composition: —NHCOO—) performed in the program TRIM [12] by a standard SRIM Monte-Carlo simulation is shown the value of penetration depth around 10 nm.

### 2.3. Growing and analysis of biofilms

Sterilized samples of 10 × 10 mm were placed in a glass vial with 2 ml of a suspension of bacteria *S. epidermidis* (33 GISK, Moscow) containing $10^8$ CFU/ml (number of bacteria in 1 ml) for 48 h at 37 °C. At the end of the incubation period the plates were washed twice in 10 mM phosphate buffer (pH 7.2). The experiments were repeated three times.

As a result, the areas formed by the one-cell thick bacteria layer (established by AFM methods) were found on the surface. The total number of bacteria on the surface and the amount of vital cells were investigated.

In the first case, the analysis was carried out by optical microscopy (video microscope Hirox KH-7700, Japan). At least 10 pictures of size 332 × 443 μm (magnification ×210 times) were captured from each sample, and the fraction of dark areas occupied by bacteria to the total area of the image was calculated.

The number of vital cells was determined by the MTS tetrazolium recovery level in the Cell Proliferation Assay system (“Promega”, USA) in accordance with the manufacturer's prescription. The optical density of MTS solutions was determined at 490 nm by a spectrophotometer PD-330 (Japan).

Preparation and analysis of biofilms were performed after one week after surface treatment and then repeated (with the same old samples) after 11 months of storage samples in dark place at room temperature.

### 2.4. Spectroscopy

The study of the Raman spectra was carried out on the multifunction spectrometer “SENTERRA” (Bruker) at a 532 nm wavelength of emitting laser.

### 2.5. Atomic force microscopy

The properties of surfaces were studied using an atomic force microscope Dimension Icon (Veeco, USA) in a nanomechanical mapping mode (PeakForce QNM). Nanoindentation was carried out at each point of the surface with a frequency of 2 kHz [13]. The obtained force curves of interaction of the AFM-probe with the surface were analyzed. The following structural–mechanical properties of the material were studied: 1) surface height; 2) adhesion force between the tip and the sample; 3) depth of penetration of the probe into the material; and 4) material stiffness (elastic modulus $E$ calculated in terms of the Derjaguin–Muller–Toporov (DMT) model).

Depending on the hardness of the surface, the probes ScanAsyst-Air (by Bruker, a nominal tip radius $R = 6$ nm, stiffness $k$, calibrated by free thermal oscillations — 0.6 N/m) or HA-FM (by NT-MDT, $R = 10$ nm, $k =$ 5.5 N/m) were used. The depth of indentation into the material was kept at a value of ~5 nm. In this case the applied probe-sample force was 2 (untreated PU), 10 (PIII $2 \cdot 10^{15}$ ions/cm$^2$), 15 (PIII $2 \cdot 10^{16}$ ions/cm$^2$), and 20 (PIII $2 \cdot 10^{17}$ ions/cm$^2$) nN. The probe radii used in DMT-model were calibrated by measuring the known stiffness of polydimethylsiloxane samples (2.5 MPa).

For measuring a tip-sample adhesion force, only soft probes ($k =$ 0.6 N/m) were used, and the force applied to the surface was small. In this case, the indentation depth was <2 nm for untreated PU, and ~0 nm for PIII treated samples.

A structural–mechanical analysis was carried out to study the samples in both undeformed and stretched static states. In the latter case the sample was stretched and fixed in a small device that was placed directly under the microscope scanner.

### 2.6. Mechanical dynamical tests

An investigation of the surface of treated samples after dynamic mechanical loading has also attracted our interest. Samples with size of 10 × 4 mm were tested for 10 min in a dynamo-mechanical analyzer DMA/SDTA861e (Mettler Toledo, Switzerland) with a frequency of 30 Hz and a 10% strain. After loading, the samples were examined by AFM.