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Research on Surgical Implant Coatings Generating Nitrogen(II) Oxide

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Abstract

The paper investigates coatings and compositions of model fluids during the dissolution of titanium oxynitride films in physiological media by infrared (FT-IR) spectroscopy and gas chromatography-mass spectrometry (GC-MS), and also atomic emission analysis. The presence of nitrogen gas in the investigated samples of the Ti−N−O biocoating and a nitrogen(II) oxide (NO) molecule, vital for a living organism, in solutions has been determined.

Keywords: biocoating, oxynitride films, IR spectroscopy, sodium nitroprusside, spectrum, chromatographic separation

INTRODUCTION

Material biocompatibility issue is key in medical materials science, in particular, during the manufacture of implants and cardiovascular stents. One of the ways to solve it is by applying biocompatible coatings onto medical products. Pure titanium that forms titanium oxide film is most often used as a bioinert material. The oxide film is stable in time, dielectric and practically does not enter into chemical reactions with biological fluids and compounds [1]. Coatings made of both titanium dioxide and titanium oxide doped with nitrogen (TiN_xO_y) with technological substitution of oxygen for nitrogen atoms are most often used in practice. The authors of [1, 2] determined that material properties were changed with increasing nitrogen gas content in the coating, such as anti-thrombogenic properties appeared and the degree of hemocompatibility increased. Nevertheless, it has not been sufficiently explored how precisely nitrogen affects the properties of thin films.

It is assumed that nitrogen oxides that are involved in numerous physiological reactions, beginning with the regulation of the cardiovascular system to bioregulation of nerve system mechanisms are liberated into a solution during contact of liquid media of the body with the implant surface. Furthermore, nitrogen(II) oxide inhibits thrombocyte aggregation. However, a high level of NO generation causes a toxic effect characterized by damage to cell structures and DNA mutation. In this regard, the issue of the determination of nitrogen(II) oxide in biological fluids during contact with the surface of nitrogen-doped implants is relevant.

The purpose of the present research was to explore Ti−N−O thin films (their possible corrosion and dissolution) during a long-term contact

with model media imitating the organism. The main task was to determine the presence of nitrogen(II) oxide in the composition of model solutions to further explore its effect on the body.

MATERIALS AND METHODS

Samples of biocoatings were produced by reactive magnetron sputtering using a UBN-200MI experimental plant (TPU, Tomsk) [2]. The 316L steel plates (10 × 10 × 1 mm) were the substrate for applying single and double sided coatings. Furthermore, to examine the films (sample 1) by IR, the coatings were applied onto monocrystalline KBr plates and to determine film elements by gas spectroscopy – onto NaCl ones. Oxygen (O_2) and nitrogen (N_2) were used as the plasma forming gas. Coating deposition parameters are Ti cathode material, chamber working pressure of 10^{-1} Pa, power of 1 kW, current of 3 A, working gas flow rate of 5 mL/min, and offset voltage from 0 V to -100 V. Ratio of gas partial pressure of N_2 and/or O_2 ($p(O_2)/p(N_2) = 1/1$), deposition time of 90 and 180 min were used. According to coatings solubility research, it was required to carry out analysis of the chemical composition of model fluids after a long-term contact of samples of coatings with the medium, gravimetric determination of the mass loss during experiments, and also macro- and microscopic analysis of the coatings before and after treatment with solvents. Solutions such as Ringer's (sample 2), of urea, hydrochloric acid, acetic acid, and lactic acid in concentrations appropriate to body conditions (6.5 g/L of NaCl, 0.42 g/L of KCl, and 0.25 g/L of $CaCl_2$; 4 g/L; 0.3 g/L; 74 %; and 1 mmol/L, respectively) were used as model fluids. The volume of solvents was determined depending on the sample area according to the recommendations of GOST R ISO 10993-12-2009. The chemical composition of the solutions was analysed on the atomic emission spectrometer ICAP 6300 Duo (atomic emission spectroscopy or AES). Solutions of model fluids were used as reference solutions [3]. The weighing was carried out with an accuracy of 0.0000 g using Acculab ALC 210 d4 electronic scales [4, 5].

In order to assess correlation properties of coatings for coronary stenting, samples of Ti–N–O films precipitated on steel were aged in Ringer's solution (the basis is a saline NaCl solution) for 300 days with regard to projections of their behaviour the biological medium (the blood stream).

The method of infrared (IR) spectroscopy (Nicolet 6700 IR-Fourier spectrometer, USA) was used to identify chemical bonds present in Ti–N–O coatings and the solution with possible diffusion process of coating elements coating therein. Infrared spectra were taken in the 400–4000 cm^{-1} range. A solid probe of the material (film sample 1) applied onto KBr, and also comparison standard were ground in an agate mortar with KBr in the 1 : 100 ratio until the formation of an indiscrete mass (tablet pressing method) and the absorption spectrum of the sample was removed. Potassium bromide does not absorb IR radiation in a wide wavelength range (250–2500 nm), which allows it to be used as a substrate for this method.

Absorption bands identification was carried out according to tables of characteristic frequencies on the ground of literature and reference data [6, 7]. In order to analyze the liquid phase, the liquid film method was used, whereby the compound drop was placed onto a plane glass surface and then was "got dried off" by a salt plate [8]. The same IR spectroscopy method explored adsorption spectra of an alcoholic solution of NaCl after contact with (the experiment was reproduced), which was required to minimize the overlapping of absorption bands in the spectrum [9]. In order to detect the C–N bond, comparison standard, *i.e.*, a compound with a nitro group, sodium nitroprusside, $(Na_2[Fe(CN)_5NO] \cdot 2H_2O$, (a salt and an aqueous solution) was used.

Analysis of nitrogen amount in coating samples and solutions was determined by gas spectroscopy using the CHNS Flash 2000 Thermo Scientific elemental analyzer, Switzerland (the Collective Use Centre, TPU). The method is based on chromatographic separation of gaseous combustion products using a packed column operating in the isothermal mode [10]. The CHNS furnace temperature is 900–1000 °C (temperature rise up to 1800 °C for several seconds during a dynamic flash). Quantitative analysis used a sample containing up to 5 mg of the coating sample (sample 1) on the NaCl crystal, and Ringer's solution after long-term ageing of the coating sample, up to 5 mL (sample 2), therein.

RESULTS AND DISCUSSION

The dissolution rate of Ti–N–O coatings in model fluids was explored in detail in [3–5]. It was determined by instrumental methods of quantitative analysis (AES and gravimetry) that the films

were chemically resistant [11] and did not change the qualitative and quantitative composition of biological fluids due to a negligible drop in the mass of samples and the trace content of surface elements (film/metal) in the solution.

Figures 1–3 give IR spectroscopic research on Ti–N–O films and experimental solutions. Figure 1 represents a typical IR spectrum of samples of coatings. Infrared spectra of coatings contain adsorption bands that correspond to vibrations of Ti–O groups at 1357 and 790 cm^{-1}, which may be explained by the presence of separate oxide and titanium nitride groups found in the unbound state [12]. A broadening of absorption bands in coatings obtained at a negative voltage shift of 100 V during magnetron application testifies the formation of an ordered crystal structure of Ti–O groups (see Fig. 1).

The presence of absorption bands in the 3000–3500 cm^{-1} region, which corresponds to the signal of valence oscillations of ON-groups of water, is typical for IR spectra of the liquid phase (see Fig. 2) [7].

An intense narrow absorption band with a maximum of 1635.6 cm^{-1} identifies N–O stretching vibrations, the presence of which is confirmed by reference data (the absorption region of N–O in the 1680–1450 cm^{-1} range) [8], and also by comparison of the IR spectrum of the analyzed samples with the spectrum of sodium nitroprusside standard, $Na_2[Fe(CN)_5NO] \cdot 2H_2O$ (see Fig. 4).

Nevertheless, IR spectra interpretation of aqueous solutions is hampered because of the strong absorption of water IR radiation [7] in regions of 3700–2800, 1800–1600, and below 1000 cm^{-1}. Solvent replacement in the experi-

Fig. 2. IR spectrum of saline (NaCl) solution before (1) and after (2) the contact with the Ti–N–O film.

mental fluid for alcohol is intended to address the issue with inaccuracies in the assessment of the absorption spectrum of dissolved substances, presumably from the Ti–N–O coating [9]. The spectrum of the alcohol solution (see Fig. 3) contains characteristic bands of O–H stretching vibrations in the 3650–3200 cm^{-1} region, those of C–H in the 3000–2840 cm^{-1} range, of C–C deformation vibrations, vibrations in the 1279–1340 cm^{-1} area, strongly-intense lines of C–O stretching vibrations near 1125–1000 cm^{-1}, middle-intense peaks below <900 cm^{-1} may be referred to deformation vibrations of OH and CH_2 groups. The spectrum also contains a weak peak at 1686.4 cm^{-1}, which similarly to in the IR spectrum of the aqueous solution of NaCl may be attributed to the valence vibrations of N–O. Reference spectra (according to the N–O bond) of dry crystalline $Na_2[Fe(CN)_5NO] \cdot 2H_2O$ and its aqueous solution have peaks of 1612 and 1634.4 cm^{-1} (see Fig. 4),
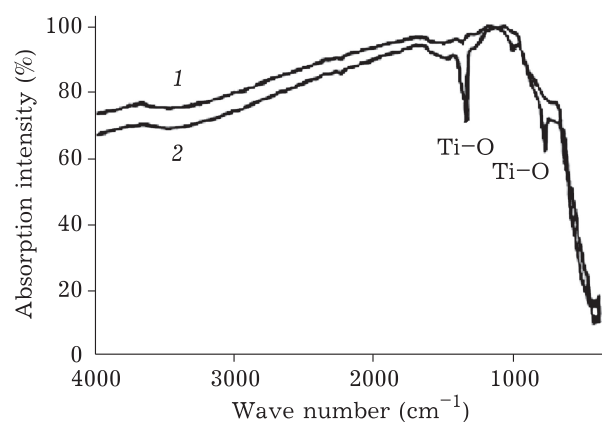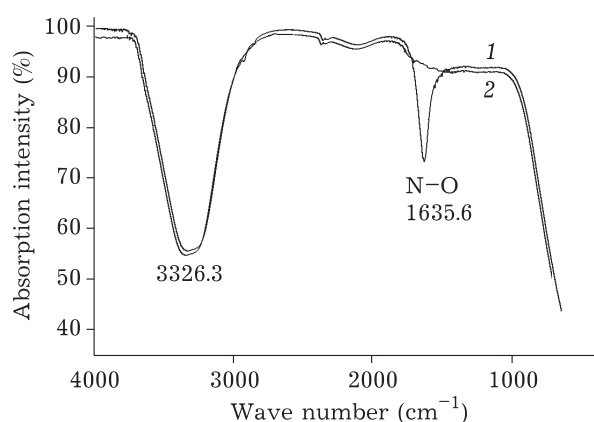

Fig. 1. IR spectrum of Ti–N–O coating at the lack of the voltage shift on the table and upon the shift of -100 V during its deposition.
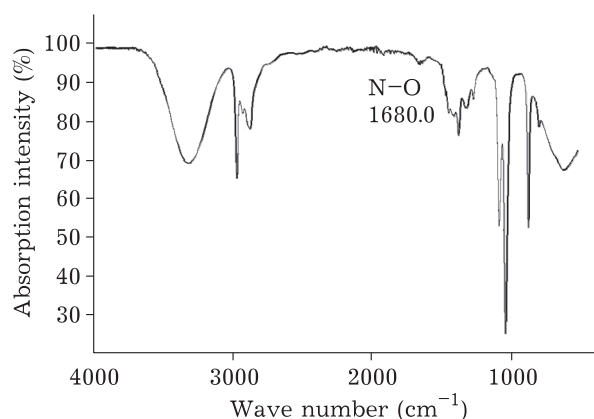

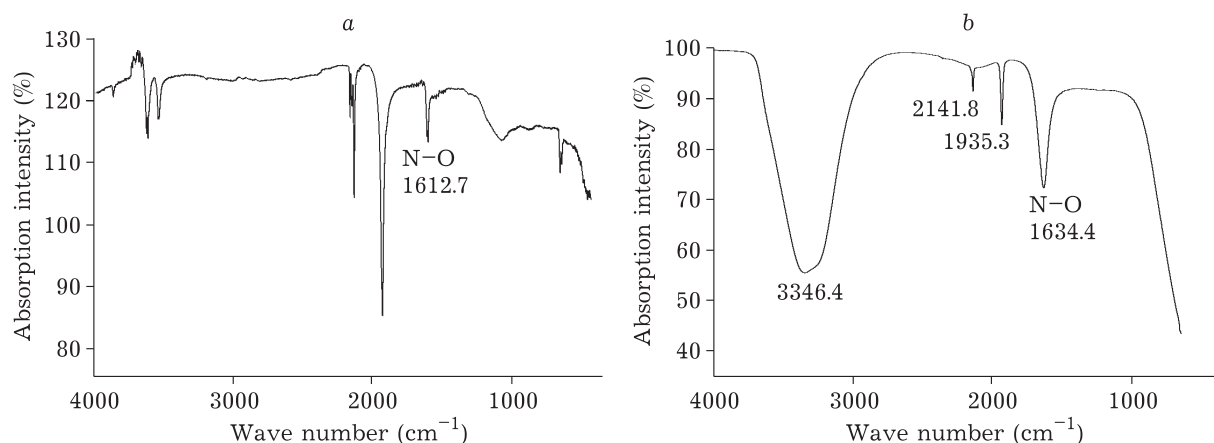Fig. 3. IR spectrum of a NaCl solution after contact with the Ti–N–O film.

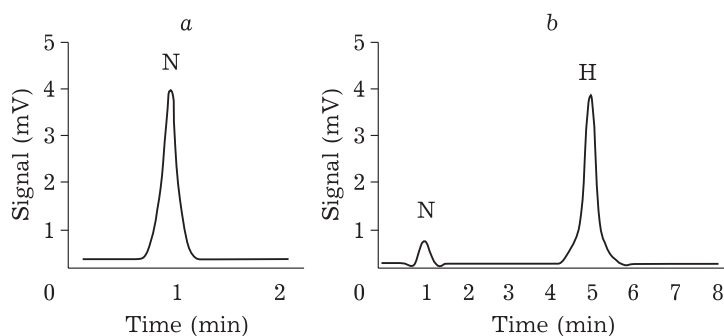Fig. 4. IR spectrum of the $Na_2[Fe(CN)_5NO] \cdot 2H_2O$ crystal (*a*) and its aqueous solution (*b*).

Fig. 5. CHNS analysis results of samples: *a* − coating (sample 1), *b* − model liquid after a long-term contact with the coating (sample 2).

respectively, which is consistent with the literature and experimental data. As demonstrated by spectral analysis, the characteristic peaks in both samples are the same. However, the width of the bands in the IR spectra of crystals is slightly lower than that of liquids, which is due to the ordered arrangement of particles in the crystal lattice. The IR spectrum of sodium nitroprusside contains absorption bands in the $3570-3730$ cm^{-1} range belonging to valence vibrations of hydroxyl groups of crystallization water. Highly intense lines for both samples in the $1900-2200$ cm^{-1} range are inherent to CN and CNO.

In this manner, as it follows from IR spectroscopy the synthesized layers of the oxide film contain the main elements, such as Ti, O, and N. The presence of spread-out peaks (Fig. 1) testifies that the Ti−N−O thin film, whereof nitrogen(II) oxide may pass into a solution during contact with fluids, is nanocrystalline or pseudoamorphous.

Resulting from quantitative analysis of nitrogen in samples 1 and 2, the following data, such

as the corrected retention time (t_R), peak width, and component percentage in the samples computed for each component, were acquired by gas spectroscopy using a CHNS analyzer. Figure 5, and also Table 1 present test data for the samples with appropriate numbers.

The experimental data demonstrate the presence of nitrogen on the surface of the samples and indirectly prove the presence of NO in model fluids. Moreover, nitrogen content in the solution is two orders of magnitude lower than that in the coating

CONCLUSION

In this manner, research results point to reagent resistance of the Ti−N−O film. Nevertheless, the revealed slight dissolution of the coating (within the error) in the liquid medium promotes the release of NO from the film. This is likely to facilitate the activation of biochemical processes in the body as a whole.

TABLE 1

CHNS analysis results of samples

Sample	Component	Retention time, min	Peak area, ODU*	Content, %
1	N	0.983	26015	8.731
2	N	0.995	10270	0.072
	H	4.758	6373734	10.320

* optical density units.

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