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Nanocomposite coatings for implants protection from microbial colonization: Formation features, structure, and properties

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ABSTRACT

A comprehensive study of the effectiveness of two-layer coatings based on PEG and silver nitrate to combat pathogenic microorganisms – the agents of implant-associated infections – was conducted. The paper deals with the features of the structural transformations that occur in the polymer matrix at the stage of deposition and formation of the thin layer, as well as when the thin layer is heat-treated. The PEG matrix was shown to promote thermal stabilization of silver nitrate, which is manifested in the presence of significant amount of oxide (AgO and Ag_2O) and undecomposed salt, in addition to silver nanoparticles, in the annealed organic layer.

1. Introduction

Bacterial infections seriously complicate the usage of implants. A foreign body significantly increases susceptibility to infection. The most common microorganisms that cause posttraumatic infections are Staphylococci, Enterobacteriaceae and Gram-negative non-fermentative bacteria [1].

The infection area developing around the implant leads to increased susceptibility to infections due to the fact that on the inert surface of the implant, the microorganisms acquire the ability to complex colonization, forming polysaccharide matrix, the so-called biofilm. The biofilm, in turn, inhibits the phagocytosis and significantly reduces the efficiency of antibiotics [2].

The emergence and spread of resistance to the most used in current clinical practice antibiotics, including carbapenems, among opportunistic Enterobacteriaceae and Gram-negative non-fermentative bacteria greatly complicates the antibacterial therapy of the infections caused by them. The actual set of antibacterial agents that are effective against Gram-negative infections agents is very limited, meanwhile only a few promising antibiotics of quite limited activity spectrum are expected in the nearest future. Most Gram-negative pathogens of nosocomial origin are characterized by multiple, extreme or even panresistance to antibacterial preparations. The resistance of Gram-negative pathogens to carbapenems caused by the production of metallo-β-lactamases (MBL) is of special epidemic importance. The MBL genetic linkage with other resistance determinants is in many cases accompanied by the development of extreme antibiotic resistance.

So far, only polymyxins retain the acceptable microbiological activity against many carbapenem-resistant nosocomial isolates of Enterobacteriaceae and Gram-negative non-fermentative bacteria; to refer to their resistance profile, the "POS" (polymyxin-only-susceptible) abbreviation was introduced. There are a number of reports about panresistant strains of *P. aeruginosa* and *A. baumannii*, resistant to colistin [3,4].

The formation of antibacterial coatings on the implants surfaces is an effective way to prevent the implant-associated infections [5]. To date, the main requirements for the design and composition of such antibacterial layers are formulated [6–8]. In addition to maintaining the necessary concentration of the antibacterial agent near the implant surface, the coating should prevent the adhesion of microorganisms (including

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dead ones) on its surface. Otherwise, with time, a biofilm might be formed that prevents direct interaction of pathogens with the antibacterial agent. The imparting of "cleansing" properties to the coatings is achieved with the usage of water-soluble polymers, which are slowly destructing in the human body biological environment [9]. It should be noted that for a number of implants, not only the suppression of antibacterial infection, but also the prevention of thrombotic complications (thrombus formation) is necessary [10], which also requires the use of water-soluble polymers.

Silver nanoparticles, although their toxicity toward the cell cultures was detected in certain cases, are an effective alternative to contemporary antibacterial agents to combat pathogenic microorganisms [11]. The analysis of the studies of silver nanoparticles toxicity shows that polyethylene glycol may be considered a good alternative to various organic stabilizers of silver nanoparticles used in industrial and medical applications, where it is essential to reduce the toxicity to human cells. Polyethylene glycol has a high solubility with respect to substances of both hydrophilic and hydrophobic character. The nanoparticles processing allows improving their biological stability and biocompatibility [12,13]. The high efficiency of PEG-coated metal nanoparticles is noted in cardiosurgery. This is due to imparting of high biocompatibility to the nanoparticles and to the prevention of platelet aggregation and adhesion by inhibiting the biochemical aggregation pathways of platelets (not due to the loss of platelets) [13,14].

It is known that PEG is commonly used as a polymer matrix in creation of systems for drug components delivery [15]. In this regard, the formation of thin layers based on PEG and silver nanoparticles on the implant surface will allow to effectively and durably combat antibiotic-resistant pathogenic microorganisms without significant toxic effects on human body cells.

This study suggests the deposition of two-layer PEG – $AgNO_3$ systems as coatings with prolonged release of silver nanoparticles. Such coatings are planned to be used when modifying the elements of the Ilizarov apparatus. The formation of silver nanoparticles in the polymer matrix will occur during the heat treatment (air sterilization for medical products). The method is easily integrated into the standard pattern of preoperative medical products preparation, implants in particular, and does not require any additional specific equipment. For comparison, we examined the coatings deposited with electron-beam dispersion of mechanical mixture of PEG and silver nitrate powders.

2. Methodology of the experiment

2.1. Methodology of forming coatings

The coatings were deposited from the gas phase formed by the exposure of the target to low-energy electron flow of 800–1600 eV energy and 0.01–0.03 A/cm^2 density. The coatings deposition process was carried out in a vacuum chamber at the initial residual gases pressure of $\approx 4 \cdot 10^{-3}$ Pa. The two-layer systems deposition is economically feasible to carry out in a single technological cycle without depressurizing the vacuum chamber. However, we carried out a two-step deposition process, which was methodologically necessary. Initially, the PEG-based layer was deposited. The next step was the depressurization of the vacuum chamber, placing clean substrates in the vicinity with the PEG-based ones, the deposition of $AgNO_3$ thin layer. This deposition pattern, taking into consideration the products dispersion distribution diagram, made it possible to form silver nitrate layers of the same thickness on all the substrates. The deposition conditions may be considered identical. The effective thickness of the layers formed was directly controlled during the deposition using a quartz crystal microbalance (QCM) and corresponded to 1 μm.

The composite coating was formed by the exposure of the mechanical mixture of PEG and $AgNO_3$ powders in a weight ratio of 2:1, respectively, to low-energy electron flow. The mass of silver nitrate in the mixture corresponded to the salt mass in the target during $AgNO_3$ deposition. The deposition of the coating was conducted for the entire period of gas phase generation.

The substrates temperature during the deposition of thin layers corresponded to the room temperature.

2.2. The material of coatings and substrates

The powders of polyethylene glycol, (PEG, $M_n = 4000$, Aldrich) and silver nitrate ($AgNO_3$, ≥99.0%, Aldrich) were used as the target material.

The substrates for the layers deposition were quartz plates during spectroscopic measurements in the UV–Vis region, NaCl plates during IR spectroscopic studies, silicon (100) single crystal plate during SEM and XPS studies, copper meshes with a deposited carbon layer during TEM studies, titanium plates during antibacterial studies.

2.3. Features of the heat treatment of the formed coatings

The heat treatment of the formed coatings was carried out in air at the temperature of 100 °C, 200 °C, and 250 °C for 30 min. The selected temperatures covered the full range of temperatures used in the heat sterilization of medical devices. The high temperature heat treatment of the coatings formed on special substrates for TEM studies is impossible. The heating process might be followed by warpage of the substrate, which is unacceptable. In this regard, the deposition and the heat treatment of thin layers were performed on NaCl substrates. Then the substrate was placed into distilled water. In water the thin layer was separated. The separated coating was carefully transferred onto a copper mesh and dried in a stream of dry warm air.

2.4. Structure and morphology studies

The X-ray structure analysis of the coatings was performed on the Bruker D8 Advanced X-ray diffractometer using the radiation source of Cu Kα ($\lambda = 1.54056$ Å), 40 kV, 40 mA.

The chemical composition of the deposited layers was determined by XPS. The measurements were conducted on the PHI Quantera II Scanning XPS Microbrobe spectrometer using the Al Kα source of monochromatic X-ray radiation ($h\nu = 1486.6$ eV). The analysis results were processed with the OriginPro software package.

Morphological thin layers studies were performed on a scanning electron microscope (SEM Quanta 200 F).

The molecular structure was studied on Vertex-70 (Bruker) IR-Fourier spectrophotometer. The scanning was performed in the range of 4000–300 cm^{-1} with 4 cm^{-1} resolution. As an internal standard, the band of stretching vibrations of C—H bonds in CH_2 groups (2920 cm^{-1}) was used. The optical density value of that band was correlated with the optical density values at 1115 cm^{-1} (stretching vibrations of C—O) and at 1720 cm^{-1} (stretching vibrations of C═O groups).

The UV–Vis spectroscopic studies were performed using the Cary-50 (Varian) spectrophotometer.

2.5. Microbiological studies

For microbiological studies, the extreme antibiotic-resistant clinical isolates of *Pseudomonas aeruginosa* 144 MBL VIM, *Klebsiella pneumoniae* K-165 MBL NDM, *Acinetobacter baumannii* 50 OXA-40, *Acinetobacter baumannii* 65 OXA-23 were selected, they are resistant to all antibacterial preparation except polymyxins. The microorganisms were secured from hospitalized patients with osteomyelitis and implant-associated infections.

Preliminarily all the samples were air sterilized at 160 °C for 60 min. The antibacterial activity of the coatings on titanium plates was tested by two-layer agar method. The sterile plate was placed surface up into a Petri dish onto the surface of Mueller-Hinton agar (MHA). Then 10 ml of melted MHA were poured as a second layer onto the plate surface (the height of the agar second layer is about 1 mm) and kept at the