RESEARCH OF ANTIBACTERIAL PROPERTIES OF THE SURFACE OF THE TITANIUM ALLOYS VT5-1 AND VT6 CONVERTED TO ANATASE TO PREVENT BACTERIAL ADHESION

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Abstract: Aim: to evaluate the effectiveness of preventing the formation of microbial biofilms on the surfaces of implants made of titanium alloys VT5-1, VT6 with a transformed surface into anatase.

Materials and methods. The study was carried out in the bacteriological laboratory of the Severodonetsk multidisciplinary city hospital from January 2019 to September 2019. The ability to prevent the formation of microbial biofilms of Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa was evaluated. The static cell adhesion method and the disco diffuse method were used. The surface of the plates was examined on a XS 6220 UCMJS05100KPA microscope. The intensity of the formation of microbial biofilms on the plates was evaluated visually by the four-cross system.

Results. On all coatings with anatase, in comparison with unmodified plates, the intensity of initial adhesion and the number of bacteria fixed on the surface of the colonies markedly decreased. On surfaces with silica crystals, biofilm formation was minimal. The altered surface of titanium alloys VT5-1 and VT6 anatase and anatase with silicon dioxide crystals does not inhibit microflora growth.

Conclusions. The transformed surface of titanium implants into anatase and anatase with silicon dioxide crystals prevents the fixation of biofilms of microbial associations.

Keywords: antimicrobial coatings, titanium implant, anatase.

1. Introduction

Infectious complications in surgery, despite the achievements of pharmacotherapy in antibiotic prophylaxis, continue to occur with the use of implants. In Ukraine, patients after endoprosthetics of large joints, patients with cancer prostheses, the prognosis after septic complications is not always favorable, treatment protocols require large material and time costs [1, 2]. The first stage in the development of an infectious complication is the formation of microbial films [3]. Since bacterial adhesion is one of the first steps in the formation of a biofilm, the creation of conditions that impede the attachment of cells to the surface can significantly slow down or completely impede its formation. A decrease in the adhesion of microorganisms on the surface of materials can be achieved due to functional polymer coatings [4, 5]. Coatings with silver content [6, 7] and biocomposite coatings with metal oxides have antimicrobial properties [8]. Recently, there have been works on the use in medicine of titanium implants with an oxidized surface in anatase [9, 10]. It is proposed to use this method of surface transformation of the anatase titanium alloy, which contains silicon dioxide crystals (patent for invention u2019 02729).

Aim: to evaluate the effectiveness of preventing the formation of microbial biofilms on the surfaces of implants made of titanium alloys VT5-1, VT6 with a transformed surface into anatase.

2. Materials and methods

For the anatase-converted surface of titanium alloys, the ability to prevent the formation of microbial biofilms of Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa is evaluated.

The study was carried out in the bacteriological laboratory of the Severodonetsk multidisciplinary city hospital from January 2019 to September 2019.

Most studies of the antibacterial properties of surfaces are carried out in bacterial suspensions in a static mode or in a flow cell; the viability of the amount of planktonic form of bacteria is estimated depending on the contact time with the modified surface. In the laboratory of the Severodonetsk multidisciplinary city hospital, it is not possible to work with open colonies of microflora, therefore, the development of bacteria in closed tubes and closed Petri dishes is evaluated. It is also taken into account that during the formation of a biofilm, it consists of up to 80 % protein matrix. Therefore, by the number of fixed matrices after autoclaving, the intensity of bacterial development in fixed biofilms is estimated.

The static cell adhesion and the disc diffuse methods are used.

In the first series of experiments, the adhesive properties of anatase are investigated. A milliary suspension of Staphylococcus aureus and Escherichia coli (1.5×108 CFU/ml) is prepared. 5 ml of each microorganism strain suspension is poured into each tube, then plates (5 plates in each series, air sterilization, t-160°, 60 minutes) from VT5-1, VT6, plates from VT5-1, VT6 with transformed in the anatase surface, plates of VT5-1, VT6 with the surface converted to anatase with the presence of silicon dioxide in the pores. The contents of the tubes are mixed 2 times a day. After 3 days, the tubes with plates are autoclaved. The surface of the plates is examined on an XS 6220 UCMJS05100KPA microscope under a magnification of 100X. The intensity of the formation of microbial biofilms on the plates is evaluated visually by the four-cross system.

In the second series, intraoperative implant contamination is simulated. Sterile plates (five plates in each series, air sterilization, t-160°, 60 minutes) from VT5-1, VT6 and plates from VT5-1, VT6 with anatase-converted surface are placed in Petri dishes on an agar-agar layer, next to the contact with the plates, the museum flora of Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa are looped. The growth of microflora is evaluated on the first, second and third days, the distance of the growth of bacterial colonies to the edge of the plates is measured with a caliper.

In the third series, the inhibitory effect of the anatase surface on the activity of bacterial substances is studied, and the disk diffusion method is used. According to the standard method, the museum flora of Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa is seeded in a Petri dish with agar-agar, sterile plates of VT5-1, VT6 with anatase-converted surface and plates of VT5-1, VT6 with transformed are placed on top into anatase surface with the presence of silicon dioxide in the pores of crystals. The plates are air sterilized, t-160°, 60 minutes. The growth of microflora is evaluated on the first day.

3. Results

After autoclaving, VT5-1 and VT6 plates shows traces of black and brown biofilms in all cases.

After autoclaving on plates with an anatase-modified surface, traces of biofilms are not detected in test tubes with St. aureus culture. In test tubes with E. coli culture, there were isolated traces of light films.

After autoclaving on plates with an anatase-changed surface with crystals of silicon dioxide, traces of biofilms are not found in all test tubes.

The results show that on all coatings with anatase, in comparison with unchanged plates, the intensity of initial adhesion and the number of bacteria fixed on the surface of colonies markedly decreased (**Table 1**). On the surfaces of anatase with crystals of silicon dioxide, the formation of biofilms is minimal.

Table 1
The intensity of the formation of biofilms on the surfaces of implants for 3 days

Implant material	St. Aurreus	Esh.coli
Titanium plate VT5-1, VT-6	++++	++++
VT5, VT-6 + TiO_2 titanium plate	+/– – –	++/– –
Titanium plate VT5-1, VT-6 + TiO_2 + SiO_2	– – – –	– – – –

In the second series, in Petri dishes with MT5-1 and MT6 plates, the colonies of cultures of St.aureus and Ps.aeruginosa touches the edges of the plates by the first day, and by the third day they begin to grow around the plates with tight contact with the edge of the implants (**Fig. 1**). Colonies of E. coli touched the plates by the first day, 10–20 mm around the plates by the third day.

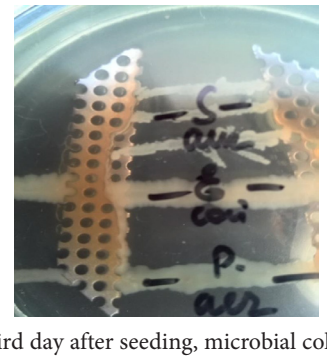

Fig. 1. The third day after seeding, microbial colonies are fixed on titanium implants after contamination

In all cases with the surface of anatase and anatase with crystals of silicon dioxide, the first days of the colony of microorganisms approach 1––2 mm to the edges of the plates, by the third day, the colonies increase in size near the plates, the shape of the implants is repeated at a distance of 1–0.5 mm, but do not touch the edges (**Fig. 2**). In the third series, in all cases of inhibition of flora growth is not noted.

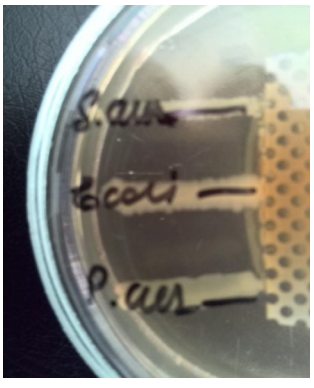

Fig. 2. The third day after seeding, microbial colonies grow near the titanium implant with the surface transformed into anatase after contamination, but are not fixed on the surface

4. Discussion

Most studies of the antibacterial properties of materials are carried out in bacterial suspensions and by the disc diffuse method, when the release of the active antibacterial form from the modified surface leads to growth retardation [6]. After diffusion into the surrounding tissue, the "active" surface is depleted and ceases to impede the development of microflora on the implant, which does not prevent the formation of biofilms in the late postoperative period. The results of suspension tests also significantly depend on the possibility of releasing active antimicrobial ingredients from modified surfaces [4], and when using implants in orthopedics, surfaces undergo significant mechanical stress during surgery, which can also lead to surface defects. Surfaces using physical properties, in particular the presence of a constant electric charge [11, 12] also have antibacterial properties. The surface of titanium converted to anatase possesses electret ability, which gives it antibacterial properties, which is confirmed by our research.

The following studies show the potential for the practical use of the antibacterial properties of titanium implant crystals converted to anatase and anatase with silicon dioxide crystals in order to prevent the fixation of microbial associations.

The altered surface of titanium alloys VT5-1 and VT6 anatase and anatase with silicon dioxide crystals does not inhibit microflora growth; such a coating resistant to microbial associations is not washed out in aqueous solutions and does not emit metal ions in sufficient quantities for antimicrobial exposure.

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