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Study of Nanostructural Coating Biocompatibility *In-Vitro*.

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ABSTRACT

The study investigates cytokines secretion ability of leukocytic cells stimulated by materials contained in implants. The stimulated cytokine-production activity in relation to the cytokines (IFNα, IFNγ, TNFα, IL-1ß, IL-1βRa, IL-2, IL-6, IL-8) was changed in the presence of and depended on the type of the studied implant. The most intensive inflammatory reaction was observed in case with metals and polyurethane, the least intensive one was registered in the presence of a nanostructural coating based on amorphous carbon and silver nanoparticles (NPs:Ag).

Keywords: medical implant, inflammation, cytokines.

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INTRODUCTION

Long service life and reliability of implants is determined by various factors which depend on specific characteristics of the used materials however infectious complications and a local inflammatory response of surrounding tissues are standard for implants of any kind and sometimes are definitive for the term of their proper functioning [1-4]. Polymers with hydrophilic coatings are the most widely used materials for stents [1, 5-7]. Use of titanic alloys seems to be a perspective trend [7-10]. Implants with carbon diamond-like coating are characterized by higher biocompatibility and a range of studies demonstrated an excellent cell compatibility of these implants [3, 7, 9].

METHODOLOGY

There were studied four types of materials. Samples of medical steel and polyurethane conventionally used for production of ureteral and endovascular stents were used as control materials. The main panel included nanostructured titanium β-alloy and NPs:Ag having the same surface area. Bioinert properties of titanium β-alloy and the stated nanostructured coating were subject to investigation for the first time. The coating was applied by a vacuum-arc technology. Especially pure graphite with addition of silver was used as a cathode. The investigated coatings had the thickness of 30 – 50 nm. A high-resolution transmission electron microscope Tecnai G2 F20 S-TWIN was used for a coating description. An ultimate composition of a coating was studied by the method of energy-dispersive X-ray spectroscopy (EDX). Analysis of the results of electron microscope investigations demonstrated that the resulting coating could be characterized as nanostructured. Figure 1 shows an image of an individual nanocluster of silver in a matrix of amorphous carbon having an ordered arrangement. The nanocluster has crystalline order. An individual crystal has a face-centered cubic lattice (FCC lattice), interplanar spacings are well compliant with the tabular data for silver. Average content of silver in the coating determined by the energy-dispersive X-ray spectroscopy method made 3 – 5 %.

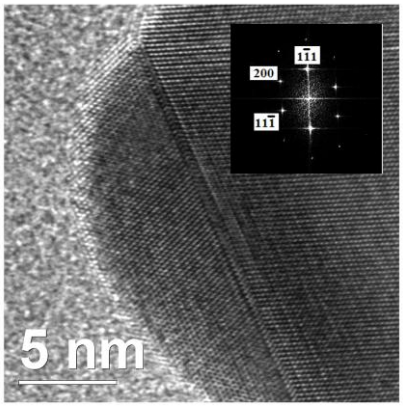

Figure 1: An electron microscope image of an individual nanocluster (high-resolution transmission electron microscopy).

The studied samples had the equal surface area of 20 mm^2. In order to study bioinertia of the investigated materials there was used a leukocytic suspension obtained from whole blood of four healthy donors having A(II) blood group. The leukocytic suspension was prepared by centrifuging in a blood bag and mixed with autoplasma in the ratio of 1:20. Cell composition and cytogram indices for the leukocytic suspension were determined automatically, their ratio didn't differ from the normal value ($p>0.05$). The ready leukocytic suspension was dispensed into 0.5 ml sterile plastic containers with the studied material which subsequently were placed into a thermostate at a temperature of 37ºC. The leukocytic suspension from each of the donors was incubated in the thermostat with ten samples of each of the materials over a period of 24 hours at a temperature of 37ºC (except for the control panel). The level of cytokines was evaluated by a solid-phase ELISA method with use of a kit produced by CJSC "Vector Best" (Moscow).

MAIN PART

After standardization of the number of leukocytes in the leukocytic suspension at the level of $15x10^9$ /l the content of granulocytes in the suspension made $6.8±0.48x10^9$/ml, lymphocytes – $6.45±0.84x10^9$/ml,

monocytes – 1.7±0.06x10^9/ml, thrombocytes – 954±108x10^9/ml. Concentration of RBC did not exceed 0.09±0.0024x10^{12}/ml, hemoglobin – 3.1±0.08 g/l, the level of hematocrit made 0.92±0.04 %. The levels of the investigated cytokines as determined in native blood of the donors were equivalent with the average population values recommended by the manufacturer of ELISA test systems and had normal distribution except for the level of the receptor antagonist of interleukin -1 beta (IL-1βRa).

There was registered a positive rise of stimulated secretion of alpha-interferon (IFNα) as compared to the initial level and the control panel in the presence of polyurethane, the increase made 4.17±0.61 pg/ml within 12 hours, 7.23±1.79 pg/ml within 24 hours, while the same indices in the control panel within the stated periods were equal to 1.18±0.31 pg/ml and 2.53±0.83 pg/ml correspondingly ($p<0.05$). In the rest study panels the level of IFNα in the tested blood samples didn't show positive difference from the initial values and its growth was in compliance with the indices in the control panel.

Stimulated secretion of gamma-interferon (IFNγ) in all panels had statistical significant difference as compared to the initial level in the donors' blood and the control level within the both terms. The maximum growth for the term of 12 hours was demonstrated in the panels with medical steel and polyurethane: 741.3±162.3 pg/ml and 813.9±211.5 pg/ml correspondingly which is positively higher as against the experimental materials. At the term of 24 hours there was registered some stabilization of IFNγ level growth rate in the mentioned panels. For titanium β-alloy the maximum increase of IFNγ secretion was observed during the second period of the experiment. The minimum ability to stimulate IFNγ secretion for the term of 12 and 24 hours was demonstrated by NPs:Ag – 55.2±15.7 and 116.3±35.8 pg/ml correspondingly which was positively lower as compared to the other study panels ($p<0.05$).

The tumor necrosis factor (TNFα) level in all study panels at the studied terms was positively higher then in the control panel: 3.12±0.74 and 4.61±0.66 pg/ml within 12 and 24 hours correspondingly ($p<0.05$). No statistically significant difference was observed in the panels with the investigated materials at the term of 12 hours ($p>0.05$). By the end of the study period the similar tendency was registered for all of the panels exclusive of NPs:Ag panel where the values of this cytokine were positively higher - 29.65±5.71 pg/ml ($p<0.05$).

Stimulated secretion of the principal anti-inflammatory cytokine (IL-1ß) was positively different in various study groups. The level of IL-1ß in the panels with medical steel, polyurethane, titanium β-alloy over 12 hours made 57.5±11.2, 44.1±8.3 and 42.6±7.9 pg/ml respectively, which was positively higher then in the panel with NPs:Ag – 12.5±4.7 pg/ml ($p<0.05$). During the next term the tendency to growth of stimulated and spontaneous (the control panel) secretion of IL-1ß persisted. The maximum level of this cytokine was registered for the panel with medical steel, at the same time there were no statistically significant differences as compared to polyurethane and titanium β-alloy ($p>0.05$). For this study term simulated secretion of IL-1ß in NPs:Ag panel was not different as against the control panel and was equal to 21.7±3.3 and 25.4±4.8 respectively ($p>0.05$).

A homotypic nature of secretion was registered for another anti-inflammatory cytokine of monocyte-macrophage origin – interleukin-8 (IL-8) (Table 4). Its level in the panels with polyurethane and metals was positively higher as against NPs:Ag and control panels at the both study terms ($p<0.01$). The level of stimulated secretion of IL-8 in NPs:Ag panel didn't differ from the spontaneous secretion of this cytokine in the control panel and was equal to 131.4±28.1 and 119.6±32.1 and 177.6±45.1 and 157.2±31.8 pg/ml correspondingly at the study terms of 12 and 24 hours, which showed no statistically significant differences ($p>0.05$).

The level of such regulatory cytokine as receptor antagonist of interleukin-1 (IL-1βRa) didn't demonstrate statistically significant variations at time of the experiment, its distribution was distinct from normal, average values are given in terms of median with specification of the 50% quartile range.

Changes in stimulated secretion of interleukin-2 (IL-2) consisted in statistically significant growth of the level of IL-2 in NPs:Ag panel as compared to the control panel within 24 hours from the start of study, the level was equivalent to 57.2±15.4 pg/ml against 27.1±7.8 pg/ml in the control panel ($p<0.05$). During the first 12 hours IL-2 level rise was registered in all study panels but no statistically significant difference as compared

to the panel with spontaneous secretion was revealed ($p>0.05$). After 24 hours from the experiment start comparison of the results of stimulated IL-2 secretion showed no statistically significant variations ($p>0.05$).

In all study panels there was registered growth of concentration of a regulatory cytokine – interleukin-6 (IL-6) as compared to the panel with spontaneous secretion ($p<0.05$), while between the study groups themselves no statistically significant differences were observed ($p>0.05$).

CONCLUSION

The most distinct inter-panel differences in the level of stimulated cytokine-production activity of the leukocytic suspension were registered in regard of IFNγ, IL-1ß and IL-8. The maximum level of production of the mentioned cytokines and IFNα was registered in the panels of medical steel and polyurethane, somewhat lower stimulated secretion was observed in the titanium β-alloy panel which is compliant with the literature data. The better bioinertia indices in titanium materials are conditioned by oxide film which is absent in case of steel. Implantation of materials from these panels will naturally result in intensive immune inflammatory reactions. Less intensive stimulated secretion of IFNγ, IL-1ß and IL-8 was registered for NPs:Ag panel, which can be connected with a membrane stabilizing action of silver nanoparticles in small concentration.

The fact of higher cytokine production activity in regard to the cytokines in charge of antibacterial, antiviral and antitumoral immune response (TNFα, IL-2) in NPs:AG panel generates interest, it can be explained by an indirect bactericidal action of silver in a small amount (insufficient to be directly cytotoxic).

Absence of growth of the levels of regulatory cytokines (IL-1βRa, IL-6) is probably connected with short terms of study, i.e. incompleteness of immune inflammatory reactions and absence of stimulation of specific cytokine-producing cells.

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