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Functional Status of Leukocytes in the Presence of Medical Implant Materials.

Nina Ivanovna Zhernakova*, Aleksandr Anatolyevich Dolzhykov, Sergey Valentinovich Shkodkin, Kseniya Aleksandrovna Bocharova, Vadim Nikolayevich Dmitriyev, Aleksandr Yakovlevich Kolpakov, Sergey Sergeyevich Manokhin, Oleg Vladimirovich Miroshnichenko, and Aleksey Vasilyevich Liubushkin

Federal State Autonomous Educational Institution of Higher Professional Education, Belgorod State National Research University, 85, Pobedy St., Belgorod, 308015, Russia.

ABSTRACT

The investigation was aimed at study of direct cytotoxicity of materials used in production of medical implants as well as of their influence on functional status of human leukocytes. Functional activity of neutrophils (FAN) and spontaneous cytotoxic activity of natural killers (SCANK) changed in the presence of and depended on the type of the investigated materials.

Keywords: medical implant, stent, inflammation.

Corresponding author

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INTRODUCTION

Being a foreign matter any implant induces start of a typical pathophysiological process, i.e. inflammation [1-4]. Duration of service life of an implant is often determined by a capacity to minimize inflammatory reactions [3, 5-8], i.e. by bioinertia towards the body tissues [1, 6, 8]. Protection of the implants by means of bioinert coatings and impregnation of their surface with antiproliferative agents are the main ways to solve this problem [9-10].

METHODOLOGY

Samples of medical steel and polyurethane conventionally used for production of stents were studied as control materials. The main panel included nanostructured titanium β-alloy, nanostructural coating based on amorphous carbon and silver nanoparticles (NPs:Ag) having the same surface area. The coating was applied by a vacuum-arc technology. Especially pure graphite with addition of silver was used as a consumable 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 object corresponded to nanostructural coating. Figure 1 shows an electron microscope image of NPs:Ag. Inclusions of darker color are silver nanoclusters with the size of 20-40 nm against more light amorphous carbon matrix background. Average content of silver in the coating determined by the energy-dispersive X-ray spectroscopy method made 3 – 5 %.

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 with ten samples of each of the materials in the thermostat over a period of 24 hours at a temperature of 37ºC (except for the control panel).

In order to determine activity of non-specific protective reactions there were investigated phagocytic activity of neutrophils (FAN) and spontaneous cytotoxic activity of natural killers (SCANK). For study of FAN a day-old growth of Staphilococcus epidermidis of 9198 strain was used. At the 30^{th} and the 120^{th} minutes of incubation a phagocytic index (FI) and phagocytic count (FC) were determined. A phagocytic count coefficient (FCC) and an index of bactericidal action of neutrophils (IBAN) were calculated. For study of SCANK a two-day-old erythromyeloblastoid cell line K-562 was used as target cells. SCANK was determined by means of a flow cytometer as ratio of a number of lyzed target cells (initial number of the target cells less number of cells after incubation) to initial number of the target cells.

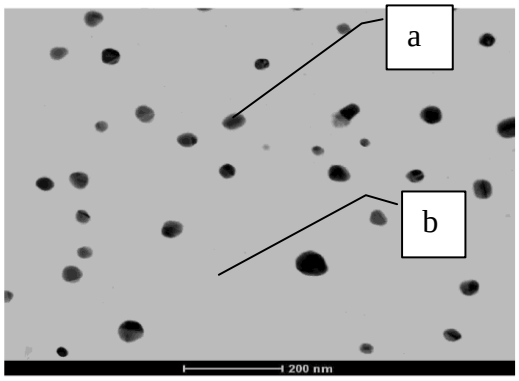

Figure 1: An electron microscope image of NPs:Ag.
a – silver nanoclusters; b – amorphous carbon matrix.

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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 %. Initial relative content of subpopulations of T-lymphocytes, FAN and SCANK tests didn't differ from normal value ($p>0.05$).

Within 12 hours after incubation start the control samples didn't demonstrate statistically significant changes in the level of granulocytes, monocytes, total number of lymphocytes and their subpopulations. No changes in FAN and SCANK were registered either. The maximum changes were observed in the panel with medical steel. They were represented by statistically significant cytolysis of granulocytes ($5.69±0.46x10^9$/ml) as compared to the initial values (6.82±0.48x109/ml) and the control panel values (6.72±0.27 x109/ml) ($p<0.05$). Cytolysis of granulocytes in this panel was accompanied by growth of functional activity both of neutrophils and natural killers, moreover statistically significant differences were obtained both for FI and FC at the both terms ($p<0.05$), no growth of FCC (1,12±0,05) and IBAN (65.4±3.46) as compared to the initial values (1.17±0.04 and 59.2±3.1) and the control panel (1.03±0.05 and 59.2±3.08) correspondingly ($p>0.05$) were registered. In this study panel appearance of neutrophils with degranulated nuclei and toxic granulosity in cyloplasma were observed.

No statistically significant cytolysis was registered in the rest of panels at this term. Functional status of nonspecific protective mechanisms was characterized by growth of SCANK, FAN and the derivative indices, but these indicators had no statistically significant difference as compared to the control group except for the panels with medical steel as it was mentioned earlier. No positive differences were observed between the studied materials. In the panel with polyurethane at the 120^{th} minute there was registered the statistically significant growth of fagocytic activity (FI120, FC120) and correspondingly decrease of FCC (81.03±3.27%, 13.47±0.81 bacteria, 0.67±0.15) as compared to the experimental materials, the mentioned indicators for NPs:Ag panel made – 67.32±4.42%, 8.56±0.29 bacteria, 1.03±0.05 ($p<0.05$). Increase of the indicators FAN at the 120^{th} minute was registered also for β-alloy (76.07±7.26%, 9.4±1.8 bacteria) however no statistically significant differences as compared to the control panel were registered ($p>0.05$). The content of lymphocyte subpopulations at this term was not changed and was no different from the control panel.

Moderate cytolysis was observed in the control samples 24 hours after the study start on the part of granulocytic cells, this was accompanied by change of FAN. Statistically significant growth of FI and FC was registered for the both time intervals (FI30 – 74.49±5.87%, FI120 – 76.59±6.91%, FC30 – 10.76±0.84 bacteria, FC120 – 11.04±1.63 bacteria) as compared to the initial data (FI30 – 63.4±4.5%, FI120 – 61.9±3.8%, FC30 – 8.83±0.69 bacteria, FC120 – 8.83±0.69 bacteria) and the control panel indices after a 12-hour period (FI30 – 64.15±4.26%, FI120 – 64.03±2.93%, FC30 – 8.63±0.69 bacteria, FC120 – 8.56±0.29 bacteria) ($p<0.05$). Indices of FCC and IBAN didn't change, growth of SCANK didn't have statistically significant differences in the study panels ($p>0.05$).

In the samples with materials cytolysis was characterized by less specificity and higher intensity as compared to the control panel, statistically significant decrease of the level of leukocytes was registered in the panels with medical steel, polyurethane and β-alloy: 9.21±0.43x109/l, 10.7±0.43x109/l, 10.75±0.65x109/l correspondingly ($p<0.05$). In comparison with the control panel the level of leukocytes in NPs:Ag panel had no statistically significant differences, the mentioned indicator in the stated panels made 13.48±1.12x109/l and 13.48±1.12x109/l correspondingly ($p>0.05$). The results of analysis of immunograms in the panels with medical steel, polyurethane and β-alloy demonstrated absence of statistically significant differences in respect of the absolute levels of monocytes, B-lymphocytes and subpopulations of T-lymphocytes as compared to the control panel as well as correlation in regard to the content of granulocytic cells and natural killers, the level of which was positively lower then in the control panel and in NPs:Ag panel ($p<0.05$). While content of NK-cells in these study panels made no significant difference 0.29±0.08x109/l, 0.34±0.12x109/l, 0.28±0.11x109/l correspondingly) ($p>0.05$), the level of granulocytes in the panel with medical steel had minimum values and showed statistically significant difference as against the panels with polyurethane and β-alloy: 3.32±0.49x109/l, 4.52±0.34x109/l, 4.56±0.25x109/l correspondingly ($p<0.05$). The group with medical steel was characterized by presence of neutrophils with toxigenic granulation, cytoplasm vacuolation and

degranulation of neutrophils nuclei. The indicators of cytolysis in NPs:Ag panel showed no statistically significant differences as compared to the control panel ($p>0.05$).

The panel with medical steel demonstrated significant suppression of FAN at the both time intervals and SCANK (FI30 – 24.02±4.08%, FI120 – 25.1±4.08%, FC30 – 3.66±2.31 bacteria, FC120 – 3.44±2.37 bacteria, SCANK – 9.08±1.75%) both compared to the other panels and to the initial values (FI30 – 61.9±3.8%, FI120 – 64.4±4.5%, FC30 – 8.83±0.69 bacteria, FC120 – 7.57±0.48 bacteria, SCANK – 35.1±2.5 %) ($p<0.05$). Statistically significant decrease of FAN (especially at the 120th minute) and SCANK as compared to NPs:Ag and control panels was registered in the panels with polyurethane and β-alloy ($p<0.05$).

CONCLUSION

Cell-mediated responses to the implants were realized through nonspecific protection mechanisms which became apparent due to change of FAN and SCANK values. At that the panels with low bioinertia indices of materials (medical steel, polyurethane and β-alloy) showed significant growth of FAN and SCANK as early as within 12 hours after incubation start which was accompanied by neutrophils cytolysis in the panel with medical steel. 24 hours after these panels demonstrated the prevailing cytolysis processes mainly due to activity of neutrophils and natural killers which was accompanied by reduction of FAN and SCANK values. In the panel with NPs:Ag cellular reactions had the similar nature but cytolysis phenomena were statistically less intensive, no FAN and SCANK suppression was observed at the studied terms. The received data allow making a conclusion that this material has better biocompatibility.

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