BIOCOMPATIBILITY OF OXIDIZED ANODICALLY TITANIUM ALLOYS

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Introduction

The most commonly used procedure for modification of surface layer of titanium alloys is anodic oxidation improving biocompatibility [1,2]. Physical and chemical properties of the produced layers depend not only of the parameters of the anodizing process, but also on the preliminary, preceding mechanical and electrochemical surface treatments [3,4]. Biocompatibility studies, in particular the cytotoxicity of anodically oxidized titanium alloys do not include the concentration of metal ions in the extract obtained from the tested samples.
The study determined the effect of extracts from titanium alloys with a modified top layer on the survival of SaO2-2 cells. In addition, tests of the concentration of metal ions in the applied extracts were carried out.

Materials and Methods

The research were carried out for surface layers produced on samples from rods of Ti6Al4V and Ti6Al7Nb alloys. The surface modifications of the samples included pre-treatment treatments: grinding (1), vibratory processing (2), mechanical polishing (3), sand blasting (4), electrolytic polishing (5), anodic oxidation (97V) (6) and steam sterilization (S) [X]. Cytotoxicity tests were performed in accordance with PN-EN ISO 10993-5 and PN-EN ISO 10993-12. Samples with a modified top layer were used on Ti6Al4V ELI and Ti6Al7Nb alloys substrates. Tests were carried put using the model cell line SaOS-2 (bone cancer, osteosarcoma, *Human Osteosarcoma cell line*, CLS, Cat. No. 300331). The MTS test was carried out, which involved determining the amount of formazan in the cells that only living cells are able to produce. As a positive control, sodium dodecyl sulfate was used, the negative control was a culture medium with fetal bovine serum. Cell survival in the test, positive and negative control groups was determined by measuring the amount of formazan formed during 72-hour cell incubation with titanium alloy extracts, sodium dodecyl suflate and fetal bovine serum. The amount of formazan was determined in absorbance studies at 490nm. In addition, microscopic evaluation of the effect of extracts on the SaOS-2 cell line was carried out. Immunofluorescence staining was used to visualize the cytoskeleteon of cells at the microtubule level by observing tubulin protein. Hoechst dye was used for imaging the nucleus. The concentration of metal ions in the extracts of titanium alloys with a modified surface layer used in cytotoxicicty and microscopic observations of cells was determined by the ICP-AED method.

Results and Discussion

In the cytotoxicity tests, the slightly different degree of interaction of their extracts with the survival of SaOS-2 cells was found. The criterion for assessment – PN EN ISO 10993-5 standard – allows to state that the produced layers and their degradation products do not cause cytotoxic effects. Microscopic examinations of SaOS-2 cells in both control and incubated with Ti6Al4V ELI and Ti6Al7Nb alloys extracts with a modified top layer showed no change in morphology and cytoskeleton organization (FIG. 1). The cells adhered evenly to the substrate and were properly flattened. At the same time lamellipodia/filopodia testify to proper migration.
Cells were also observed in the division phase confirming proliferation. Analysis of the chemical composition of titanium alloy extracts with a modified surface layer indicates slight differences in the concentration of elements (Tab. 1). High concentrations of vanadium ions in extracts of Ti6Al4V, niobium ions in Ti6Al7Nb alloy extracts and low concentration of aluminum ions for both alloys should be emphasized.

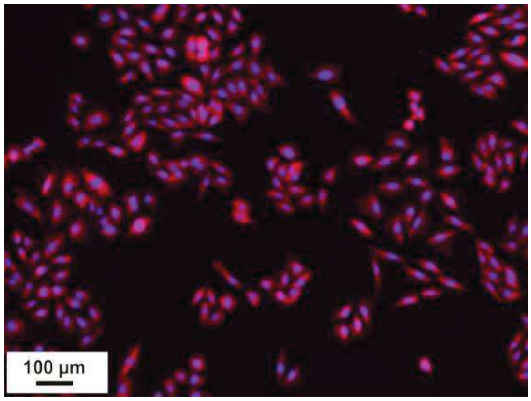

FIG. 1. Image of cells subjected to incubation with extracts from Ti6Al4V alloy of modified surface 1/3/4/97V/S

TABLE 1. Concentration of metal ions in Ti6Al4V and Ti6Al7Nb alloy extracts with modified Surface layer

Alloy	Modification method of surface	Concentration of metal ions, ppm							
		Ti	σ_{SD}	Al	σ_{SD}	V	σ_{SD}	Nb	σ_{SD}
Ti6Al4V	1/2/3/4/97V/S	1.05	0.09	0.65	0.10	0.91	0.05	–	–
	1/3/4/97V/S	1.10	0.10	0.65	0.10	0.80	0.03	–	–
	1/3/4/5/97V/S	1.20	0.10	0.67	0.10	0.81	0.03	–	–
	1/2/5/97V/S	1.33	0.20	0.57	0.09	1.12	0.09	–	–
Ti6Al7Nb	1/2/3/4/97V/S	1.04	0.20	0.45	0.05	–	–	1.22	0.11
	1/3/4/97V/S	0.99	0.06	0.40	0.05	–	–	1.01	0.09
	1/3/4/5/97V/S	1.00	0.10	0.44	0.09	–	–	1.11	0.05
	1/2/5/97V/S	1.30	0.20	0.54	0.02	–	–	1.20	0.09

Conclusions

Produced by anodic oxidation layers and their degradation products do not cause cytotoxic effects.
Cells of the SaOS-2 line after incubation with the Ti6Al4C and Ti6Al7Nb alloys extracts with a modified surfaced showed no change in morphology and organization of them. Due to the lack of reference levels of safe, non-cytotoxic effects on cell cultures, concentrations of titanium alloy degradation products obtained in the work concentration can be considered as the reference level.

References

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