COMPREHENSIVE BIOLOGICAL EVALUATION OF BIOMATERIALS USED IN SPINAL SURGERY

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Introduction
It seems that commonly used biomaterials are sufficiently well characterized allowing the responsible selection of biomaterial for the purpose of spine surgery. However, a thorough analysis of available publications indicates the difficulty of comparing them due to the variety of research techniques and experimental conditions. In addition, there is no information on tissue responses observed at the molecular level, allowing for the identification of mechanisms involved in cell response to exposure to the used biomaterial. For this reason, we have decided to perform a comprehensive biological evaluation of representatives of commonly used biomaterials (Ti6Al4V alloy) and polymer (PEEK Optima).

Materials and Methods
The biomaterials of our interest were materials most commonly used in spine implantation. These are Ti6Al4V ELI alloy and PEEK Optima. Compliance with the relevant ISO/ASTM standards of biomaterials used was confirmed by the certifications provided by manufacturers indicating as implantable implant material. Additionally Ti6Al4V alloy prepared by the selective melting with electron beam technology (Ti6Al4V ELI-EBT) was used in this study [1]. The samples were prepared in the form of discs (8 or 16 mm in diameter) of 3 mm thickness. The surfaces of the samples were finished according to the standards used for the production of implantable medical devices. Metal samples were additionally etched and passivated. The final stages of sample preparation, including washing, double-sleeve packaging and steam sterilization, were conducted in a clean zone. The biological evaluation of the examined biomaterials consisted of the following methods: XTT cytotoxicity test [2], micronucleus test [3], surface colonization by bacteria [4], thrombo-compatibility [5], proteome profile [6], transcriptome profile [7]. As a biological material *E. coli* bacterial cells, EA.hy926 line of endothelial cells and Saos-2 line of osteoblasts were used.

Results and Discussion
The extracts obtained from the studied samples showed no significant cytotoxic and genotoxic characteristics for both types of cells in comparison to control culture.
Both Ti6Al4V ELI and PEEK Optima exhibited high resistance to bacterial colonization while lacking the cytotoxic properties in relation to bacteria. In contrast, Ti6Al4V ELI-EBT was significantly more susceptible to microbial colonization.
Blood platelets adhered to the surface of the studied biomaterials and underwent differentiated activation on these surfaces. On the other hand, flow cytometry analysis showed that the contact with titanium alloys samples results in five times higher spontaneous aggregation of platelets remaining in the whole blood and only two-fold increase in the case of contact with PEEK Optima, although fraction of activated platelets in whole blood, evaluated with specific antibodies, shows only a small increase in comparison to control.
Both transcriptome and proteome profiles show very significant changes in gene and peptide expression in both osteoblasts and endothelial cells resulting from contact with the studied samples. These changes concern numerous cellular metabolic pathways and ongoing analysis of the possible effects of these changes are underway.

Conclusions
Comprehensive biological analysis of biomaterials commonly used in spine surgery shows that the results of standard cytotoxicity and genotoxicity tests, although consistent with the available in literature, do not provide complete information about the suitability of these materials. It should be noted that the porous structure of Ti6Al4V ELI-EBT promotes the microbial colonization of this biomaterial. On the other hand, all investigated biomaterials produce significant changes in the expression of genes and peptides, which can be of great importance in the appropriate selection of materials for personalized medicine.

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