DOES SURFACE STRUCTURING OF METALLIC MATERIALS AFFECT THROMBOCOMPATIBILITY?

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Introduction

Blood contact with biomaterial surface results in blood platelet adhesion, activation, and aggregation on the surface [1]. Growth factors released during platelet activation have a positive effect on osseointegration. Laser structuring of the surface of metallic biomaterials allows control of osteoblast proliferation and affects the formation of microbial biofilm on the surface [2]. The question arises what the impact of laser surface structuring on the thrombocompatibility of this surface is. The aim of this research was to determine the thrombocompatibility level of laser-modified metallic materials surfaces. Thrombocompatibility was assessed both on the surface of the laser-structured materials and also in the whole citrated blood after the contact with the tested surfaces.

Materials and Methods

Four types of materials were subjected to the thrombocompatibility test: AISI 316L, Ti6Al4V, Ti6Al7Nb, CoCrMo. The samples surfaces were structured differently: Series A - surface after machining (Ra 1.1 ÷ 1.2 μm), Series B - grinded surface (Ra 0.5 ÷ 0.8 μm), Series C and D - two different types of laser modification. Human citrated blood from healthy volunteers who did not take any drugs that affect platelet function within two weeks prior to the donation has been used. The studies have been approved by the Local Bioethics Committee at the Medical University of Lodz (no. RNN/46/06/KB 21.02.2006).

To assess the adhesion, aggregation and activation of platelets on the surface of tested materials, the samples were incubated for one hour with whole blood at 37°C, ensuring a gentle blood flow. Then, it was fixed in 2.5% glutaraldehyde and dehydrated in increasing concentration of ethyl alcohol (50%, 60%, 70%, 80%, 90%, absolute alcohol). This way prepared samples were sputtered with a 5-nanometer gold layer and imaged using a scanning electron microscope. Pictures were taken at random locations on each surface tested. Platelets were counted using the ImageJ software. The study of aggregation and activation of platelets in the whole citrated blood was examined using a flow cytometry technique. After one-hour incubation of citrated blood with the test samples, blood platelets were labeled with three fluorescence labelled antibodies: CD61-PerCP recognizing the CD61 platelet marker present on the platelet surface (β subunit of GPIIb/IIIa integrin); CD62-PE recognizing the CD62 receptor (P-selectin); FITC-PAC-1 recognizing the active form of fibrinogen receptor (GPIIb/IIIa). Blood samples were then fixed with CellFix reagent and analyzed on BD Accuri C6 or FACS ARIA flow cytometer (Becton Dickinson).

Results and Discussion

A contact of blood platelets with the artificial material surface triggers platelet activation, which is evidenced by platelet shape change. The stages of platelet activation, according to the Goodman's classification [3], were analyzed: from single no-activated platelets, through dendritic and dendritic-spread platelets (medium activation) to spread and completely spread with diffuse hyaloplasm (high activation). Platelet aggregation on the surfaces of the tested materials was assessed by counting small aggregates (up to 10 platelets) and large aggregates (above 10 platelets).

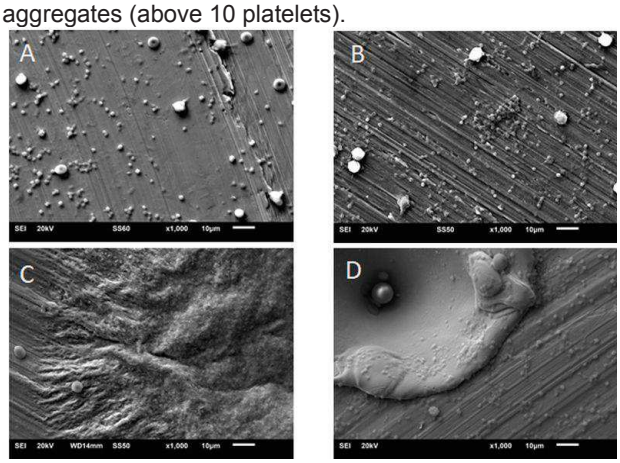

FIG. 1. SEM images of the surfaces with adhered blood platelets : A) AISI 316L, B) Ti6Al4V, C) Ti6Al7Nb, D) CoCrMo.

Flow cytometry studies allowed to determine the population of the aggregated platelets and the degree of platelet activation evidenced by the expression of two platelet activation markers.

Conclusions

Surface laser treatment within the D series for each of the tested materials promotes low activation of adhered blood platelets (the highest number of non-activated platelets) in comparison to the A, B and C series, especially for Ti6Al7Nb alloy.

The smallest number of aggregates in whole blood were observed after contact with grinded surfaces (Series B) of Ti6Al4V alloy, followed by the second titanium alloy Ti6Al7Nb.

All materials with C type modification caused the highest activation of platelets in comparison with other types of modifications. The lowest blood platelet activation in the whole blood was observed after the contact with B and D type modified materials surfaces.

Acknowledgments

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