IN VITRO INVESTIGATION OF THE PLATELET AND LEUKOCYTE ACTIVATION ON DIFFERENT CRYSTALLOGRAPHIC SURFACES OF SILICON CARBIDE

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Introduction

We aimed to reduce anticoagulant usage of patients against the thrombotic effects of artificial cardiovascular implants. The implants made of superior materials, which bring hemocompatible and mechanical properties together, can be tailored to achieve this goal. A promising candidate would be a high-strength ceramic with mechanical reliability, chemical inertness and biocompatible surfaces. In this research, cytocompatibility and hemocompatibility of a silicon carbide (SiC) single crystal were investigated in terms of crystallographic structure and surface atomic arrangement. Different crystallographic structures (single- and poly-crystal), surface terminations (Si-rich and C-rich) and polymorphs (4H and 6H) of SiC were used in order to reveal the interactions between blood content and material surface. The experiments were elaborately designed to control all parameters affecting the platelet activation and endothelial cell proliferation.

Materials and Methods

Advance diffractometer was used for the crystallographic phase composition analysis of ceramic specimens; the diffracted intensity versus 2θ is recorded by a detector. Surface roughness values of the single crystals were measured by atomic force microscopy (AFM). In order to gain a deeper insight on wettability, the static and dynamic contact angles of the samples were measured with an optical contact angle measurement and a contour analysis system.

Cytotoxicity of the specimens were analyzed by the live-dead staining assays for human umbilical vein endothelial cells (HUVECs) and blood cells. In order to examine the blood cell activation in terms of platelets and white blood cells, human peripheral blood mononuclear cells (PBMC) were isolated from the whole blood. Cell behaviors on the single crystal ceramics were analyzed under the dynamic flow conditions besides of the static cell culture conditions, in order to simulate physiological blood flow conditions of blood vessels. CD62P marker was used to investigate activated and adhered platelets by using ELISA assay. Visualization of the structure and interaction of blood cells with ceramic specimens were performed by using scanning electron microscopy (SEM).

Results and Discussion

AFM measurements verified that both monocrystalline and polycrystalline ceramic samples have comparable average surface roughness varying between 2 and 2.7 nm. According to contact angle measurements, all samples were found to be hydrophilic. However, there are differences in the degree of wettability. Cell culture results showed that the platelet activation and fibrin formation on single crystalline SiC are significantly lower than its polycrystalline form (FIG. 1). This can be attributed to the similar semiconducting properties of single crystalline SiC surface and blood proteins which lowers the surface charge transfer [1]. However, the surface charge transfer is not properly limited to prevent complete cell activation as in pure hemocompatible endothelial surface.

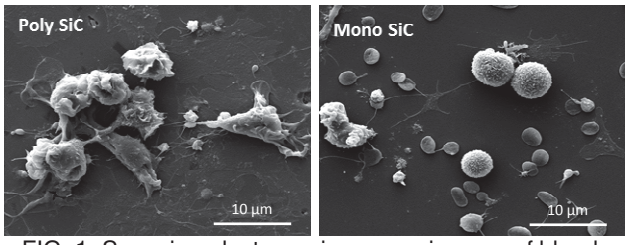

FIG. 1. Scanning electron microscopy images of blood cells on the single crystalline SiC (left) and polycrystalline silicon carbide (right) surfaces.

We also observed that Si-rich surface triggers the platelet activation and protein adsorption reproducibly and slightly more than C-rich surface, while 4H and 6H polymorphs showed no significant difference (FIG. 2).

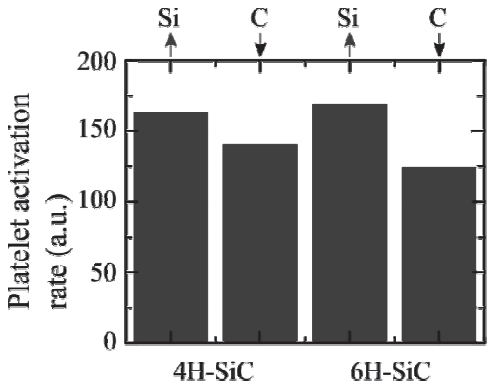

FIG. 2. Platelet activation rate depending on different crystallographic orientations and surface terminations of single crystalline silicon carbide.

Conclusions

The aim of the present work was to find out the influencing factors on the hemocompatibility. Polycrystalline and singe crystalline silicon carbide were examined by means of HUVECs and blood cells in order to investigate and compare their blood compatibility and cytocompatibility as a result of biological, morphological and physical characterizations. We conclude that, different crystal structures and surface terminations of silicon carbide play an important role on the cell responses. These findings provide vital information for the improvement of ideal surfaces for cardiovascular applications.

References

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