BIOLOGICAL EVALUATION OF ZrC COATING ON STAINLESS STEEL 316L: ARE ZrC-COATINGS SUITABLE FOR CARDIO-VASCULAR APPLICATIONS?

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Introduction

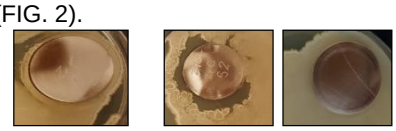
Bioreactivity in terms of the interaction of cells and tissues with the implant, occurs directly on the material surface. When using devices for cardiovascular applications, the reactions of the body to the implanted material such as protein adsorption, bacterial adhesion, phagocytosis, haemolysis, platelet activation and adhesion, and biodegradation must be considered. The healing process after implantation can be accelerated by functionalizing implant surfaces. The most known methods for surface functionalization are surface treatment with extracellular proteins (fibrinogen, collagen, albumin), the immobilization of growth factors or the treatment with the extensive cell plasma as well as whole blood. Blood as the first component of contact with the implant is crucial for solidification and healing of the implant site [1-3]. In the presented study the bacterial tests and cytotoxycity tests by using L929 were performed on the ZrC-coatings. Additionally, to evaluate the hemocompatible character of the new coatings platelets activation was executed.

Materials and Methods

ZrC-coatings were deposited on polished 316L steel substrates by using pulsed and reactive magnetron sputtering. Through spraying the Zr target in the C_2H_2 atmosphere under different flow rates, coatings with carbon content of 61 and 75 at% were deposited.
The bacteria from the American Collection of Pure Cultures Staphylococcus aureus ATCC 25922 were applied. The antimicrobial activity of the coatings was examined according to the SN 195920 standard. The susceptibility testing of the surfaces of the coatings to microbial adhesion was performed in accordance with ISO 22196: 2011.
Cytotoxicity of the specimens were analyzed by the live-dead staining assays for mouse fibroblasts (cell line L929). For the platelets activation, human peripheral blood mononuclear cells (PBMC) were isolated from the human whole blood. Platelets activation on ZrC-coatings in terms of their structure, adhesion and morphology was evaluated by scanning electron microscopy (SEM).

Results and Discussion

All coatings appeared to be bacteriostatic (FIG. 1). A low number of bacterial cells adhering to their surface was detected (FIG. 2).



61% C 75% C steel 316L
FIG. 1. Bacteriostaticity coatings.

In the present study it was found that after adsorbing of fibrinogen on the ZrC-coatings, the number of adhered bacteria increased. Probably, besides of bacterial adhesions, which in the contact with the protein initiate specific ligand-receptor processes, additionally the deficiency of coagulase or other proteins in the structure of bacteria causes them to join in those places that complement these deficiencies [4].

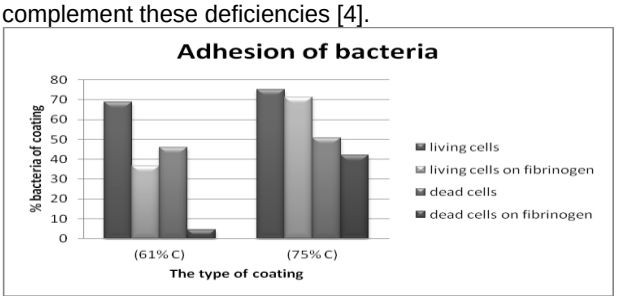
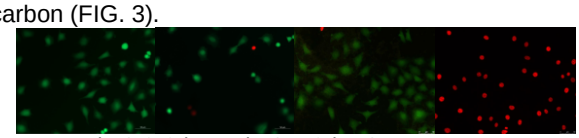


FIG. 2. Adhesion of bacteria.

Examination of the cytotoxic effects of ZrC coatings showed that only coatings with a carbon content of 61% were biocompatible with mouse fibroblasts. Cytotoxic effects were observed on samples containing 75 % of carbon (FIG. 3).



A (ZrC 61%) B (ZrC 75%) C D
FIG. 3. 24h: A, B Live/Dead Staining L929 (20x), C, D Live/Dead Staining L929 TCPS Control.

The cytocompatible coating wass selected fot platelets activation tests. The number of attached blood cells (platelets and leukocytes) on the surface is significant lower than the glass control which indicates hemocompatible character of the coating (FIG. 4). We did not observe any morphologically-destructed cells, which confirms the cytocompatible properties.

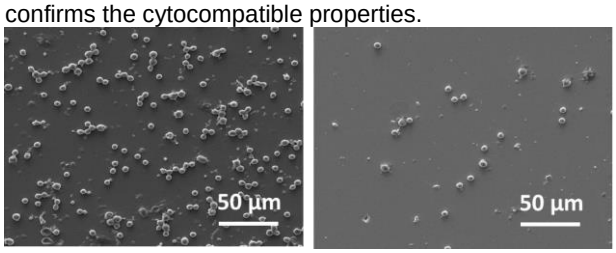


FIG. 4. SEM images of leukocytes and platelets on the lass control (left) and ZrC (right) after 4h incubation.

Conclusions

The study of ZrC-coatings showed that their use in cardiovascular applications can be considered in the future. The ZrC-coatings containing 61 % of carbon content showed the favourable biological properties. These coatings showed cytocompatible character to mouse fibroblasts and did not affect platelets activation, which indicates their hemocompatible properties. Such coatings could improve cardiovascular devises in terms of their stability, anticorrosion character, bactericidal properties and enhanced hemocompatibility.

References

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