INVESTIGATION AND VIZUALIZATION OF THE CELLS GROWN ON CERAMIC COATING BY ELECRTON MICROSCOPY TECHNIQUES

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Introduction
Biocompatibility is a crucial property to be tested for all novel biomaterials. The first step in standard approach is *in vitro* cell culture on the top of tested material and subsequent analysis of the proliferation and viability of the cells after defined time of incubation. Various colorimetric assays together with confocal microscopy are applied to evaluate cells behaviour after contact with biomaterial. In the current work we propose application of focused ion beam-scanning electron microscope (FIB-SEM) tomography as a supplementary technique to typical biocompatibility studies. It enables to investigate the interface between cell and biomaterial at the cross-section [2].

Materials and Methods
Ceramic coating on biomedical titanium alloy Ti6Al7Nb was deposited by micro-arc oxidation (MAO) in the electrolyte containing calcium acetate and sodium phosphate. MG-63 cells were cultured on the top of obtained coatings. Prior to electron microscopy investigation cells were fixed, dehydrated and gold sputtered. Microscope NEON CrossBeam 40EsB (ZEISS) was used to perform FIB-SEM tomography, 3D reconstruction was done by ImageJ 1.44p software. Details concerning coating deposition, cell culture and microscopic investigation are described in Ref. [2].

Results and Discussion
With careful selection of process parameters it is possible to obtain rough and porous coatings containing crystalline hydroxyapatite (HA) in the outer layer by MAO. Elongated crystals of HA, with the length of 300±50 nm and width of 45±10 nm, provided high surface area for cells growth and promoted filopodia formation. MG-63 cells observed with SEM were well spread with the multiple cytoplasmic projections at the surface of the tested coating.
FIB-SEM tomography is the technique that combines cutting of the sample by gallium ions with imaging of exposed surface with secondary or backscattered electrons. Therefore, it is frequently called 'slice-and-view'method [1]. Such approach enables simultaneous sectioning of delicate cell and hard ceramic coating. Collection of about 180 images was used for 3D reconstruction and visualization of the interface between cell and ceramic coating. It presents excellent adaptation of the cell to the rough surface.

Conclusions
Scanning electron microscopy provides an opportunity to investigate cells morphology and behaviour in relation with biomaterial topography. Whereas FIB-SEM tomography allows a detailed investigation of cell-material interactions in the analyzed sample volume.

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References
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