# COMPREHENSIVE EVALUATION OF THE BIOLOGICAL PROPERTIES OF SURFACE-MODIFIED TITANIUM ALLOY IMPLANTS

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**Introduction**
Recent efforts in the field of implantology have highlighted the significance of modifying implant surface topography and biomaterial composition to improve their biocompatibility. Titanium and its alloys are commonly used as biomaterials for orthopedic, dental or neuro-surgical applications. Even though titanium based implants are typically expected to last ten years or more, their longevity is not assured and the lack of integration into the bone for long-term survival often occurs and leads to implant failure. Therefore, a planned modification of the surface of the alloys is strived to obtain a highly biocompatible coating with a strictly defined structure and architecture.

**Materials and Methods**
In order to fabricate titania nanotube coatings (TNT) on the surface of Ti6Al4V substrates, the electrochemical anodic oxidation method was used. I$^{st}$ generation titania nanocoatings were produced using an aqueous electrolyte solution - 0.3% HF and different anodizing voltage values: 5V (TNT5), 15V (TNT15) and 40V (TNT40). Samples were structurally and morphologically analyzed. They were also characterized in terms of wettability and mechanical properties. Biocompatibility of biomaterials was assessed on the basis of the degree of integration of MG-63 osteoblasts-like, L929 fibroblasts and adipose derived mesenchymal stem cells (ADSC) cultures on their surface *in vitro*. In the separate experiments, we investigated the effect of the tested nanocoatings on the proliferation level of MG-63 osteoblasts-like or L929 fibroblasts co-cultured with adipose-derived mesenchymal stem cells. MTT (mitochondrial enzyme activity) assays were used to evaluate tested specimen's influence on the cell proliferation after 24 and 72 h.

**Results and Discussion**
The goal of the presented study was to optimize the production of titania-based biomaterials with high porosity and defined nanostructure, which supports the cell viability and growth. We assessed the bioactivity of amorphous titania coatings of different nanoarchitectures (nanoporous, nanotubular and nanosponge-like) (TNTs), produced on the surface of Ti6Al4V alloy by electrochemical oxidation. Cell adhesion is more difficult on smoother surfaces due to the smaller actual surface than in the case of rough substrates. In the presented studies, the modification caused an increase in surface roughness and studies using fibroblasts and osteoblast correlate with the results of AFM causing an increase in cell proliferation with an increase in the $S_a$ parameter.

Regarding the examination by SEM, it was observed that ADSC cells had a typical spindle shape and grew evenly on the entire surface of the nanocoatings. Importantly, ADSCs formed filopodia, which effectively attached the cells to the scaffolds surface despite its hydrophobic nature. It can be concluded that the nanoporous surface is favorable for ADSCs. ADSCs cells cultured on the scaffolds alone or co-cultured with MG-63 osteoblasts also produced extracellular matrix thus functionalizing the nanocoatings.

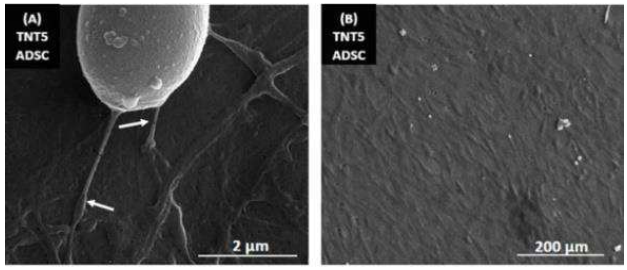


FIG. 1. Scanning electron microscopy (SEM) images showing adipose derived human mesenchymal stem cells (ADSC; A-B). Arrows in image A indicate filopodia attaching ADSC to the surface. The type of sample and scale of the images are shown in the figures as indicated.

**Conclusions**
The results of our experiments proved that the nano-porous surface is favorable for ADSC, which produced huge amounts of extracellular matrix when they were cultured on the scaffolds alone or co-cultured with MG-63 osteoblasts. The number of osteoblasts seeded and cultured with ADSCs on TNT5 surface after 72h culture almost doubled when compared with unmodified scaffold and rose by 30% when compared with MG-63 cells growing alone.

**References**
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