TITANIA NANOTUBES AS SCAFFOLDS WITH OPTIMAL NANOTOPOGRAPHY FOR ADIPOSE-DERIVED STEM CELLS

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Introduction

Since bone tissue is composed of various components in nanoscale, a surface, which mimics such structural hierarchy and provides cues at the nanoscale may further improve the response of cells on the surface to enhance bone formation and improve long term stability of the implant. Thus far, nanosurfaces have not been implemented for joint implants, however, many studies are currently directed towards understanding how nanoscale topographies on Ti/Ti alloy surfaces can improve implant integration. Some nano-topographies that have been explored in the research are nanofibers [1], nanopores [2] and nanotubes (TNT) [3]. Studies have shown that Ti/Ti alloy surface with a nanotopography promotes fibroblast and osteoblast cells adhesion and proliferation and creates an ideal environment for osteogenesis.

TNT (titania nanotubes) fabricated on the surface of titanium alloy using an anodization technique, provide a template with a hierarchy similar to that of natural tissue and have been shown to alter cellular functionality on the surface similar to that of natural tissue. However, the optimal size of nanosurfaces to promote cell adhesion, proliferation, and differentiation is still disputed. Because stem cells are important in the healing process, it is essential to study the response of stem cells on these nanostructured surfaces in vitro.

In all tissues of the body, stem cells become activated when an injury occurs and are recruited to the injury site to aid in the tissue repair process. When a biomaterial is implanted, the body reacts similar to an injury and stem cells are recruited to the implant site. Since, stem cells play an important role in tissue repair in the body, their interaction with biomaterials is critical for the long-term success of medical devices. Adipose-derived stem cells (AD-MSC), which are mesenchymal stem cells obtained from adipose tissue, have been identified as a putative population of multipotent stem cells, easily accessible, and available in large numbers. This fact makes them an attractive source for studies on evaluation of stem cell interaction with biomaterials. To date, very few studies have investigated the adhesion, proliferation, and differentiation of AD-MSC on TNT surfaces. Such research is the goal of our scientific activities.

Materials and Methods

In order to fabricate titania nanotube layers (TNT) on the surface of Ti6Al4V substrates, the electrochemical anodic oxidation method was used. The first generation nanotubes were fabricated using an aqueous electrolyte solution - 0.3% HF and different anodizing potential values (5 - 40 V). Afterwards, studies were conducted to determine the adhesion and proliferation rate of adipose-derived mesenchymal stem cells, cultured *in vitro* on oxide scaffolds on the surfaces of titanium implants. The degree of adhesion and proliferation of AD-MSC cells was evaluated using MTT (mitochondrial enzyme activity) after 24 and 72 hours. The above studies were carried out using a commercially available line of unmodified human AD-MSC cells. Biocompatibility of biomaterials was also assessed on the basis of the degree of integration of MG-63 osteoblasts and L929 fibroblasts cultures on their surface *in vitro*. Additionally, on the nanocoatings' surfaces a co-culture of (a) AD-MSC cells with L929 fibroblast cells, (b) AD-MSC cells with MG-63 osteoblast cells were formed. The degree of adhesion and proliferation of cell co-cultures was evaluated using MTT in various time variants (after 24 and 72 hours). The above studies were supplemented by the morphology analysis of AD-MSC, L929 fibroblasts and MG-63 osteoblasts cells, using scanning electron microscope (SEM).

Results and Discussion

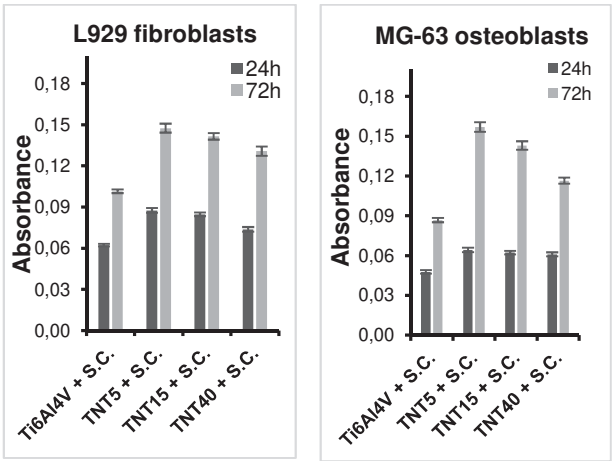

FIG. 1. Murine L929 fibroblasts and human MG-63 osteoblasts proliferation (after 24h, 72h) on the surface of Ti6Al4V and Ti6Al4V/TNT5-TNT40 samples enriched with AD-MSC, detected by MTT assay. The absorbance values are expressed as means ± SEM of four independent experiments.

Based on the results obtained from the MTT test, with an increase of incubation time more AD-MSC cells, L929 fibroblasts, as well as MG-63 osteoblasts proliferated on the surfaces of all tested biomaterials. Ti6Al4V/TNT nanocoatings provoked a significant increase in cells proliferation compared to the reference Ti6Al4V alloy. As it can be seen in FIG. 1, the nanoporous scaffoldings are also conducive to the interaction between different cell types.

Conclusions

On the basis of the obtained results, we observe that the nanoporous surface of implants is a conducive substrate for the integration and proliferation of AD-MSC cells, fibroblasts, osteoblasts, as well as cellular co-cultures, and thus promotes future bone formation.

Acknowledgments

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