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Growth and Differentiation of Mesenchymal Stem Cells on Titanium Alloy Ti6Al4V and Novel Beta Titanium Alloy Ti36Nb6Ta

Authors : Eva Filová, Jana Daňková, Věra Sovková, Matej Daniel

Abstract : Titanium alloys are biocompatible metals that are widely used in clinical practice as load bearing implants. The chemical modification may influence cell adhesion, proliferation, and differentiation as well as stiffness of the material. The aim of the study was to evaluate the adhesion, growth and differentiation of pig mesenchymal stem cells on the novel beta titanium alloy Ti36Nb6Ta compared to standard medical titanium alloy Ti6Al4V. Discs of Ti36Nb6Ta and Ti6Al4V alloy were sterilized by ethanol, put in 48-well plates, and seeded by pig mesenchymal stem cells at the density of 60×103/cm2 and cultured in Minimum essential medium (Sigma) supplemented with 10% fetal bovine serum and penicillin/streptomycin. Cell viability was evaluated using MTS assay (CellTiter 96® AQueous One Solution Cell Proliferation Assay;Promega), cell proliferation using Quant-iT™ ds DNA Assay Kit (Life Technologies). Cells were stained immunohistochemically using monoclonal antibody beta-actin, and secondary antibody conjugated with AlexaFluor®488 and subsequently the spread area of cells was measured. Cell differentiation was evaluated by alkaline phosphatase assay using p-nitrophenyl phosphate (pNPP) as a substrate; the reaction was stopped by NaOH, and the absorbance was measured at 405 nm. Osteocalcin, specific bone marker was stained immunohistochemically and subsequently visualized using confocal microscopy; the fluorescence intensity was analyzed and quantified. Moreover, gene expression of osteogenic markers osteocalcin and type I collagen was evaluated by real-time reverse transcription-PCR (qRT-PCR). For statistical evaluation, One-way ANOVA followed by Student-Newman-Keuls Method was used. For qRT-PCR, the nonparametric Kruskal-Wallis Test and Dunn's Multiple Comparison Test were used. The absorbance in MTS assay was significantly higher on titanium alloy Ti6Al4V compared to beta titanium alloy Ti36Nb6Ta on days 7 and 14. Mesenchymal stem cells were well spread on both alloys, but no difference in spread area was found. No differences in alkaline phosphatase assay, fluorescence intensity of osteocalcin as well as the expression of type I collagen, and osteocalcin genes were observed. Higher expression of type I collagen compared to osteocalcin was observed for cells on both alloys. Both beta titanium alloy Ti36Nb6Ta and titanium alloy Ti6Al4V Ti36Nb6Ta supported mesenchymal stem cells' adhesion, proliferation and osteogenic differentiation. Novel beta titanium alloys Ti36Nb6Ta is a promising material for bone implantation. The project was supported by the Czech Science Foundation: grant No. 16-14758S, the Grant Agency of the Charles University, grant No. 1246314 and by the Ministry of Education, Youth and Sports NPU I: LO1309.

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