

Cell Biological Investigations on Bioactive Surfaces

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INTRODUCTION: The surface properties of an implant material influence strongly the cellular behavior at the interface. The general goal is the control of the tissue physiology by creating bioactive surfaces [1]. In our study we examined differently modified surfaces for the stimulation of human mesenchymal stem cells (hMCS) to differentiate to osteoblastic cells in vitro. Polished titanium dishes were functionalized by amino (-NH₂) or carboxyl (-COOH) groups assuming that the different surface charges affect the cellular response.

METHODS: Polished titanium discs (Ti-P) (grade 2, R_a 0.19µm) were coated with an about 50-100 nm thin layer of plasma polymerized allylamine (Ti-PPA, NH₂-group) or acrylic acid (Ti-PPC, COOH-group). These films were prepared by pulsed low pressure microwave discharge plasma (2.45 GHz, 500 W, p=50Pa and 700 W, p=20 Pa, respectively). hMSC (6000 cells/cm², Lonza) were plated on the modified titanium surfaces and cultivated in MSCBM medium under basal and osteogenic conditions. Spreading (cell area in µm²) of PKH26-stained cells [3] was measured using confocal microscopy (LSM 410, Carl Zeiss). Quantitative real time RT-PCR assays were performed for alkaline phosphatase (ALP), collagen 1 (Col), bone sialo protein (BSP), osteocalcin (OCN), Runx2, and monitored in triplicate using an ABI PRISM® 7500 Sequence Detection System. Gene expression values were calculated by the comparative $\Delta\Delta C_T$ -method and normalized to Ti-P.

RESULTS:

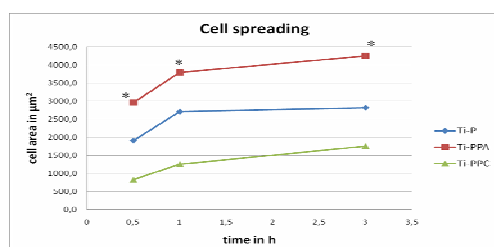


Fig. 1: Initial spreading phase of hMSC in basal medium (0,5-3 h). Cells spread significantly faster on Ti-PPA compared to Ti-P and Ti-PPC (n=40/time, U-test, *p≤0,01).

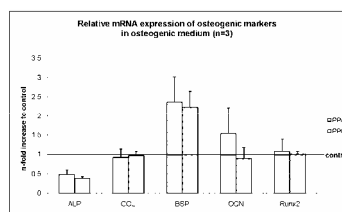


Fig. 2: mRNA expression for osteogenic differentiation markers after 3d, compared with cells on Ti-P. Note the increase of BSP and OCN.

Amino-functionalization of titanium (Ti-PPA) considerably improves cell spreading as an initial cellular effect (Fig. 1). In osteogenic medium the mRNA expression of BSP rises on both the PPA- and PPC-surfaces, whereas OCN increases on PPA only (Fig. 2). Under basal conditions the mRNA expression of ALP and Col is increased 2-fold and of Runx2 1,5-fold after 3 days on Ti-PPC compared to Ti-PPA (Data not shown).

DISCUSSION & CONCLUSIONS: The bio-activation of titanium with positively charged amino-groups improves initial steps of the cellular contact to the material surface. Concerning the stimulation of osteogenic differentiation both surfaces are able to affect the expression of osteogenic markers, depending on the culture conditions. Negatively charged functional groups appear to stimulate early mRNA differentiation markers under basal conditions, whereas osteogenic stimulation advances late differentiation markers, like BSP and OCN, after 3 days (Fig.2).

REFERENCES: ¹R. Langer, D.A. Tirrell (2004) *Nature* **428**:487-92. ²B. Finke, A. Ohl, K. Schröder B. Nebe, C. Bergemann, K. Liefeth, J. Rychly, F. Lüthen (2006) *Biomol. Eng. in press*. ³F. Lüthen, R. Lange, P. Becker, J. Rychly, U. Beck, B. Nebe (2005) *Biomaterials* **26**:2423-40.

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