Superiority of plasma chemistry over surface topography-characteristics of human osteoblast-like cells

Henrike Rebl[1], Birgit Finke[2], J. Barbara Nebe[1]*
[1]*University of Rostock, Biomedical Research Centre, Dept. of Cell Biology, Rostock, 18057, Germany*
[2]*Leibniz Institute for Plasma Science and Technology (INP), Greifswald, 17489, Germany*
henrike.rebl@med.uni-rostock.de

Introduction: Both, surface roughness and chemical modifications can influence the interaction of osteoblasts with titanium (Ti) surfaces [1]. We have observed that osteoblastic responses to smooth titanium can be improved by a positively charged surface [2, 3]. In this report plasmachemical treatments of topographically modified Ti and their effects on cell morphology are introduced.

Materials & Methods: Titanium disks (Ti, cp, grade 2), with different roughness: Polished (Ti-P: Ra=0.045 µm), machined (Ti-M: Ra=0.315 µm), and corundum blasted (Ti-CB: Ra=4.14 µm) were used and subsequently plasmachemically functionalized with a thin film (d≤0.1 µm) of microwave plasma polymerized allylamine (PPAAm). In addition, collagen I (Col) was immobilized on PPAAm via the bifunctional linker polyethyleneglycoldiacid (PEG DA) or glutardialdehyde (GDA). Human osteoblast-like cells MG-63 (ATCC) were plated onto the Ti specimens and cultivated in serum-free DMEM at 37°C and 5% CO_2. Cell shape was analyzed by scanning electron microscopy (SEM). The initial cell adhesion (5 min) was characterized by flow cytometry (FACSCalibur). Actin filament organization was observed microscopically (LSM).

Results & Discussion: Plasma-chemical modification with PPAAm enormously improves cell ingrowth into the structured surfaces. The morphology of osteoblasts demonstrates an extremely flattened phenotype on allylamine-modified surfaces and the cells seem to merge with the topography of the surface. Interestingly, we found that not only the cells but also the actin fibers are aligned along the grooves and ridges except for the PPAAm coated surface. Here, cell growth is not directed due to the dominance of the plasma chemistry, thus the cells and their actin fibers can overcome the grooved structure. Modification of the surfaces with PPAAm considerably improves the initial adhesion of osteoblasts. In addition, higher surface roughness enhances this adhesive effect. Cell adhesion is increased nearly 2-fold on both structured Ti surfaces (Ti-M PPAAm, Ti-CB PPAAm) compared to Ti-P PPAAm. In contrast, collagen I immobilization improves the cell adhesion only slightly in this initial phase. Altogether, it is striking that the positive charges seem to be dominant over the typical extracellular matrix protein collagen concerning initial cell adhesion. The PPAAm-layer is attested to be long term stable and sterilizable via gamma irradiation. We hypothesize that the treatment of implant surfaces with PPAAm is a promising method for improving cell-material-interaction.

Acknowledgment: HR and BF were sponsored with the kind support of the BMBF Germany Pilot Program Campus PlasmaMed (13N11183).

References

1. X Liu, JY Lim, HJ Donahue, R Dhurjati, AM Mastro, EA Vogler, Biomaterials, 2007, 28, 4535–4550.
2. J.B. Nebe, F. Luethen, B. Finke, C. Bergemann, K. Schroeder, J. Rychly, K. Liefeith, A. Ohl, Biomol Eng, 2007, 24, 447-454.
3. H. Rebl, B. Finke, R. Ihrke, H. Rothe, J. Rychly, K. Schroeder, B.J. Nebe, Adv Eng Mat 2010, 12, 356-364.