THE ADHESION AND GROWTH OF HUMAN OSTEOBLAST-LIKE MG 63 CELLS IN CULTURES ON TITANIUM MODIFIED WITH GOLD MICROPARTICLES AND POLY(ETHYLENE IMINE)

Martin Parizek[1], Tomas Base[2], Martin Hruby[3], Vera Lisa[1], Lucie Bacakova[1]

[1]Institute of Physiology, Acad. Sci. CR,
1083 Videnska Str, 142 20 Prague 4-Krc, Czech Republic;
E-mail: Parizek.M@seznam.cz, lucy@biomed.cas.cz
[2]Institute of Inorganic chemistry, Acad. Sci. CR, 250 68 Husinec-Rez 101,
E-mail: tbase@iic.cas.cz
[3]Institute of Macromolecular chemistry, Acad. Sci. CR,
2 Heyrovsky sq., 162 06 Prague 6;
E-mail: mhruby@centrum.cz

Abstract

Metallic materials are indispensable for construction of surgical implants, particularly those designed for load-bearing application, such as the bone-anchoring parts of big joint replacements. For good osteointegration, long-term function, durability and also mechanical and chemical resistance of the implant, the physical and chemical properties of the material surface are of a great importance. These properties can be favorably influenced by coating the bone-anchoring parts of the implants with appropriate biocompatible and bioactive films. Therefore, in this study, we have investigated the adhesion and growth of human osteoblast-like MG 63 cells in cultures on titanium substrates coated with films made of gold microparticles and/or poly(ethylene imine) (PEI).

Gold microparticles were chosen for good biocompatibility of gold and absence of its cytotoxicity, which has been proved by numerous studies performed in vitro and in vivo [1,2]. When deposited on the material surface, these microparticles provide this surface with microstructure, which has been reported to enhance the osteogenic differentiation of bone-derived cells. On the other hand, the material surface microroughness has dual effect on the cell adhesion, spreading and proliferation – some studies reported the enhancement, other the reduction of these events (for a review, see [3,4]). This suggests that not only the size of the microscale irregularities, but also their shape should be taken into account. Therefore, in our study, gold microparticles were used in the form of plates or polyhedral crystals [5]. These microparticles were deposited on square samples of Ti (1x1 cm, thickness 1 mm) and annealed with a hydrogen flame.

As for PEI, this polymer has been used as precursor base layer for further functionalization of metallic substrates, particularly with polyelectrolyte multilayer films [6] or biomolecules such as gelatin, hyaluronan

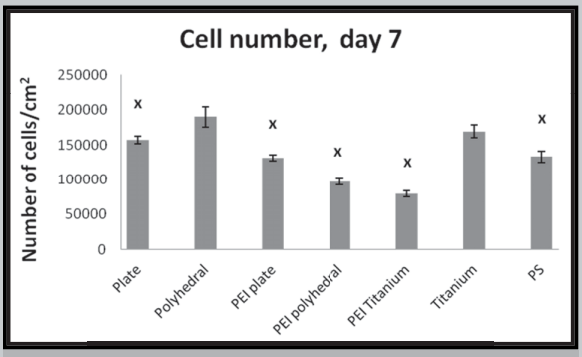

FIG. 1. Number of human osteoblast-like MG 63 cells on day 7 after seeding on titanium modified by gold microplates (Plate), gold polyhedral crystals (Polyhedral), titanium modified by gold microplates with PEI coating (PEI plate), gold polyhedral crystals with PEI coating (PEI pohyhedral), titatium with PEI coating (PEI Titanium), pure titanium (Titanium). As a reference material, standard cell culture polystyrene dish (PS) was used. Mean ± SEM from 3 independent samples for each experimental group. ANOVA, Student-Newman-Keuls method. Statistical significance: x: p≤ 0.05 in comparison with Polyhedral.

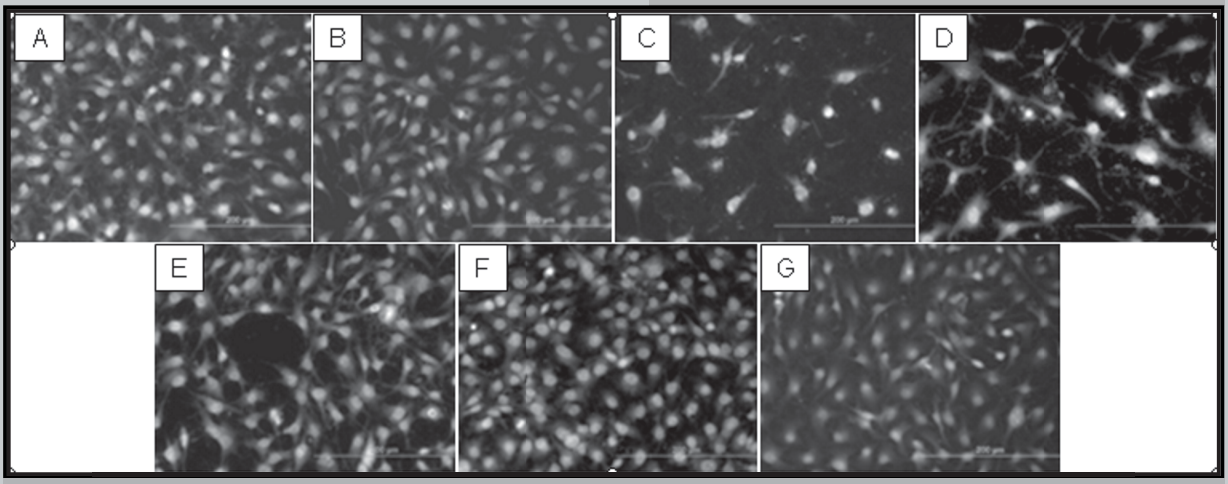

FIG. 2. Morphology of human osteoblast-like MG 63 cells on day 3 after seeding on titanium modified by gold micrplates (A), gold polyhedral crystals (B), gold microplates with PEI coating (C), gold polyhedral crystals with PEI coating (D) and PEI coating (E). As reference materials pure titanium (F) and standard cell culture polystyrene dish (G) were used. Stained with Texas Red C2-maleimide and Hoechst #33342. Microscope Olympus IX 51, obj. 20, digital camera DP 70. Bar = 200 μm.

or chitosan [7,8]. Other reason for the PEI deposition was creation of an intermediate layer which would compensate the differences in mechanical properties (e.g., hardness, toughness, specific weight) between a metallic implant and bone tissue. PEI was deposited either on pure or on gold microparticle-coated Ti samples.

The materials were sterilized with 70% ethanol (1 hour), inserted into 24-well polystyrene plates (well diameter 1.5 cm; TPP, Switzerland) and seeded with human osteoblast-like MG 63 cells (30 000 cells/well, i.e. 17 000 cells/cm^2). Each well contained 1.5 ml of a medium DMEM with 10% of fetal bovine serum and 40 μg/ml of gentamicin. On days 1, 3 and 7 after seeding, the cell number and morphology were evaluated. For evaluating the cell number, the cells were trypsinized and counted in Bürker hemocytometer. For evaluating the cell morphology, i.e. the cell shape and the size of cell spreading area, the cells were fixed with 70% ethanol (-20°C, 10 min) and stained with a combination of fluorescence dyes Texas Red C2-maleimide, which stains the cell membrane and cytoplasm, and Hoechst #33342, which stains the cell nuclei. The microphotographs of cells were taken using an Olympus IX 51 microscope equipped with a DP 70 digital camera, and the cell spreading area was measured on these pictures using a software Atlas (Tescan, Brno, Czech Rep.)

One day after seeding, the highest number of initially adhered cells was found on the surface modified by gold polyhedral crystals. This trend was the same on days 3 and 7 after seeding (FIG.1,2). However, the cell number on Ti modified with gold plates was significantly lower than on Ti with polyhedral crystals. Nevertheless, the numbers of cells on Ti samples coated with gold microparticles without PEI were significantly higher than on PEI-coated samples. Also the cell spreading areas were significantly larger on the samples without PEI. The cells on the samples without PEI were mostly polygonal, while the cells on PEI-coated samples were of star-like appearance, i.e. with multiple long protrusions (FIG.2). This is in accordance with findings published by other authors, documenting cytotoxic effects of PEI, particularly that of a high molecular weight [6], which was also used in our study (m.w. 750 kDa). Nevertheless, this cytotoxicity was considerably reduced by further functionalization of PEI with biomolecules, such as gelatin, hyaluronan or chitosan [7,8].

Thus, it can be concluded that the modification of titanium plates by gold microparticles supported the adhesion and growth of MG 63 cells. In this context, the polyhedral crystals were more advantageous than plates. The effects of PEI coatings on cell behavior need further investigation.

[Engineering of Biomaterials, 116-117,(2012), 149-150]

Acknowledgements

This study was supported by the Grant Agency of the Czech Republic (grant No. P108/10/1106).

References

[1] Gannon C.J., Patra C.R., Bhattacharya R., Mukherjee P., Curley S., J. Nanobiotechnology 6: 2, 2008.
[2] Nagesha D., Laevsky G.S., Lampton P., Banyal R., Warner C., DiMarzio C., Sridhar S., Int. J. Nanomedicine 2: 813-819, 2007.
[3] Vagaska B., Bacakova L., Filova E., Balik K. Physiol. Res. 59: 309-322, 2010.
[4] Vandrovcova M., Bacakova L.. Physiol. Res. 60: 403-417, 2011.
[5] Base T., Bastl Z., Slouf M., Klementova M., Subrt J., Vetushka A., Ledinsky M., Fejfar A., Machacek J., Carr M.J., Londesborough M.G.S. J. Phys. Chem. C 112: 14446-14455, 2008.
[6] Brunot C., Ponsonnet L., Lagneau C., Farge P., Picart C., Grosgogeat B. Biomaterials 28: 632-640, 2007.
[7] Zhong X., Lu Z., Valtchev P., Wei H., Zreiqat H., Dehghani F. Colloids Surf B Biointerfaces 93: 75-84, 2012
[8] Liu Z.M., Lee S.Y., Sarun S., Moeller S., Schnabelrauch M., Groth T. J. Biomater. Sci. Polym. Ed. 21: 893-912, 2010.