Effect of different concentration of Ti in hydrocarbon plasma polymer films on the adhesion, proliferation and differentiation of human osteoblast-like MG63 cells

Vandrovcova M[1], Grinevich A[2], Bacakova L[1], Lisa V[1], Shukurov A [2], Slavinska D [2],Biederman H [2]

[1] Department of Cell Growth and Differentiation, Institute of Physiology, Academy of Sciences of the Czech Republic, 142 20 Prague 4-Krc, Czech Republic
[2] Department of Macromolecular Physics, Faculty of Mathematics and Physics, Charles University, 182 20 Prague 8, Czech Republic

Titanium improves adhesion, growth, proliferation and differentiation of bone cells, but there is only little information about influence of different titanium content on cell behaviour. The aim of this investigation was to study effect of different titanium content on osteoblast-like MG63 cells. Titanium/hydrocarbon plasma polymer films were prepared by DC magnetron sputtering of titanium target in a mixture of two gases: *n*-hexane and argon. Concentration on titanium was controlled by adjusting the argon/*n*-hexane ratio in the working gas. This determines the emission of Ti from the target partially covered with carbonaeus C:H deposit (target poisoning). Samples with Ti concentrations ranging from zero to 24 at.% were prepared. Samples were sterilized with 70% ethanol, inserted into 12-well cell culture polystyrene plates and seeded with human osteoblast-like MG 63 cells (European Collection of Cell Cultures, Salisbury, UK). Each well contained 30,000 cells (i.e., approximately 8,000 cells/cm^2) and 3 ml of a medium DMEM, supplemented with 10% of fetal bovine serum and gentamicin. The cells were cultured at 37 °C in a humidified air atmosphere containing 5% CO_2. The cell population densities on days 1, 3 and 7 after seeding, cell spreading area on day 1, formation of focal adhesion plaques, and concentration of talin, osteocalcin and ICAM-1 were compared with the reference cell culture materials (i.e., microscopic coverslips and polystyrene dishes). Samples with 10 at.% of Ti had the most pronounced influence on the cell behaviour. On day 1 after seeding, the highest number of cells was found on this surface in comparison with other samples and controls. On the contrary, on day 7 after seeding the cell number on the sample with 10 at.% of titanium was significantly lower in comparison with the control and other tested samples. It is probable that the cells cultured on the samples with 10 at.% of Ti adhered better and entered the differentiation program earlier than the cells cultured on the other samples. This assumption is supported by an enzyme-linked immunosorbent assay (ELISA) which revealed that the concentration of talin, a focal adhesion protein associated with integrin adhesion receptors, tended to be higher in cells on samples with 10at% of Ti (as well as other samples with lower Ti concentrations up to 12 at%) in comparison with the samples with higher Ti content (15-24 at.%) and control materials, although this difference wan not statistically significant. The production of **osteocalcin**, a marker of osteogenic cell differentiation, showed an opposite tendency – higher concentrations of this glycoprotein were obtained on samples with a higher Ti content (12, 15 and 18 at.%). The concentration of **ICAM-1**, a marker of cell immune activation, was similar on all tested groups.

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