Hydroxyapatite Surface Nanoscaled characterization and Electrical Potential Functionalization to Engineer Osteoblasts Attachment and Generate Bone Tissue

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Statement of Purpose: In spite of the high success in understanding of human cells interaction with bioimplants positioned in the human organism there are still biocompatibly problems. These often are connected with incapability of the eligible human cells to be attached to the implant surface. Following the general adhesion theory attachment of the cell to the bioimplant is particularly controlled by an electrostatic force/electrical potential contributing interaction between the cell and the biomaterial. The electrical potential to the surface of the biomaterial could be supplied by the both external sources and the surface itself. The research is directed to explore both possibilities to functionalize the surface of the insulator biomaterial, hydroxyapatite (HAP) having a wide practical applications was selected as an example.

Methods: The HAP ceramic specimens were supplied from the European Commission project PERCERAMICS (NMP3-CT-2003- 504937). To engineer the surface potential due to the external source a non contact 3D technology [1] was employed. The 6 MPa of the hydrogen gas pressure was applied to the specimens, computing simulation evidenced the protons of HAP were repositioned around oxygen in a direction from the surface to the bulk, that resulted the surface provision with the negative charge/potential. To estimate alteration of the potential the noncontact gentle technique detected photoelectron emission (soft ultraviolet prethreshold mode) from the surface nanolayer. An increment of the electron work function (φ) was an index on potential alteration.

The value of φ characterizes a minimal energy that is necessary to supply to an electron to escape it from the solid during photoelectron emission. When the surface acquires the negative charge, φ increases. To measure φ, the current (I) of the emitting electrons should be detected in dependence on the photon energy (E). The condition I(E)=0 corresponds to E=φ.

To explore biological effect attachment and proliferation of the osteoblast cells were studied. SAOS-2 human osteoblasts (ATCC Cat No. HTB-85™; LGC Standards, Teddington, UK) were cultured and were added to each specimen in separate wells in an incubator. Experiments were terminated by removal of the cell suspension and washing in phosphate buffered saline to remove non-attached cells. Attached cells were then fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.3, dehydrated in alcohol and hexamethyldisilizane and further gold-sputter coated for scanning electron microscopy (SEM) using a Jeol lv3500 microscope. The observed specimens were statistically selected on a base of the Smirnov criteria (significance level 0.05).

To explore the surface of HAP as the source of the electrical potential one assumed that an intensity of the local electrical field (at the micro/nano scale) of the insulator depends of its surface morphology. Atomic force microscope was in use, the Kelvin probe mode was employed. Directions of mesenchymal stromal cell pull differentiation were studied to meet their correlation with electrical field localization parameters.

Results: The values of φ of the HAP specimens increased with extension of exposure in hydrogen atmosphere. This evidenced that processing in high pressure hydrogen deposited a negative electrical charge/potential on the specimen surface.

The number of the attached osteoblasts increased 10 times, when the increment of φ succeeded +0.18 eV.

An auto correlation function on the voltage distribution over the specimens surface was calculated on a base of Kelvin probe measurements. The correlation length (l) correlated with the surface geometrical irregularity and had a quasi linear positive influence on bone tissue fabrication.

Conclusions: 1. HAP surface electrical potential could be engineered due to both exposure at high pressure hydrogen atmosphere and surface morphology modification.

2. Functionalization of the HAP surface due to engineering of its electrical potential enhances both attachment of osteoblasts and bone tissue fabrication.

3. The achieved results are in favor of the new horizons to functionalize insulator biomaterial surface. To verify this further experiments could be done with a wide range of insulator biomaterials.

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