Nanoscale biomaterial interface modification for advanced tissue engineering applications

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Abstract. Recently, various stem cells, including mesenchymal stem cells (MSCs), have been found to have considerable potential for application in tissue engineering and future advanced therapies due to their biological capability to differentiate into specific lineages. Modified surface properties, such as composition, nano-roughness and wettability, affect the most important processes at the biomaterial interface. The aim of the present work is to study the stem cells’ (MSCs) adhesive potential, morphology, phenotypical characteristics in in vitro tests, and to distinguish between the different factors influencing the cell/biomaterial interaction, such as nano-topography, surface chemistry and surface free energy.

1. Introduction

The modification of an interface between the surface of biomaterials and the biological environment is a very important task for the modern tissue and cell engineering methods. The most intensively developed technologies are methods combining biomaterial engineering with biological substances, such as various growth factors, proteins and collagens [1-3]. Functional coatings deposition is an effective way of surface modification that provides direct control of the stoichiometry, impurity elements, functional groups and surface charges [4,5]. As the characteristic size-scale of cellular receptors interaction is a few 100 nm, the surface relief modifications should be also controlled at a nanometer-submicron level [6,7]. Studying the stem cells’ (MSCs) adhesive potential, morphology, phenotypical characteristics and distinguishing between the different factors influencing the cell/biomaterial interaction in in vitro tests is thus of great interest.

2. Materials and methods

In the present study, the samples were glass substrates (Petri dishes), uncoated and oxide-coated (Al₂O₃, ZrO₂ (MS) magnetron-sputtered and Ta₂O₅ (EB) e-beam-evaporated) with different roughness.
parameters – 20, 200 and 400 nm. The Al₂O₃ (MS) coatings deposition was performed in a high-vacuum pumping system with base pressure of about 10⁻³ Pa. The main process parameters were: magnetron discharge power 1 – 8 kW, oxygen source power 1 kW, deposition rate 8 μm/hour [8]. The Ta₂O₅ films evaporation process was carried out at initial vacuum of 7×10⁻⁴ Pa, operational mode vacuum of 3×10⁻³ Pa, anode current of 50 mA and calculated evaporation power of 350 W [9]. The deposition rate under these conditions was 28 nm/min.

The surface roughness was measured by means of a Hommel T-2000 profilometer. The surface topography was investigated by means of XPS and XRD. The X-ray photoelectron spectroscopy measurements were carried out using an ESCALAB MkII (VG Scientific) electron spectrometer at a base pressure in the analysis chamber of 5×10⁻⁸ Pa (1×10⁻⁶ Pa during the measurement), using an Al Kα X-ray source (excitation energy hν=1486.6 eV).

Other parameters, such as surface free energy (SFE), its polar and dispersion components and fractional polarity were determined by means of the Wu and Owens-Wendt-Rabel-Kaelble methods. The contact angles were measured on a Kruss K12 Tensiometer at a temperature of 20 °C [9].

Adhesion is an integral index of the cells’ structural and functional state reflecting their functional activity. Therefore, we started the in vitro experimental studies by investigating the adhesive potential of bone marrow cells (BMcs) cultured on glass uncoated and oxide-coated substrates with different roughness parameters. The MSCs phenotypical characteristics were determined by a FACS Calibur cytofluorimeter using fluorochromal monoclonal antibodies to CD73, CD106, CD44 structures.

3. Results and discussion

The dependences between the conditions of deposition and treatment of the coatings and the BMs adhesive potential were statistically and significantly different depending on the substrate materials. The best results were obtained on the glass and glass/Al₂O₃ (MS) surfaces. A statistical difference was observed between the cells’ adhesive and survival parameters depending on the surface roughness in the range 20 – 400 nm. In the same range, the cell adhesion decreased with the increase of the surface roughness parameters. Figure 1 shows the dependence between the cell adhesion (percents) and the roughness parameters for various substrate materials.

The structure of the Al₂O₃ (MS), ZrO₂ (MS) and Ta₂O₅ (EB) thin films was investigated by means of XPS and XRD. The X-ray diffraction profiles of as-deposited Al₂O₃ (MS), ZrO₂ (MS), and Ta₂O₅ (EB) coatings demonstrated an amorphous nature (no peaks were observed). It is always difficult to adjust the various parameters involved in thin film deposition to achieve tailored surface chemistry, stoichiometry and surface states of the coatings, these being important for the cell/biomaterial response. We also analysed the structure of the Al₂O₃ (MS) and ZrO₂ (MS) oxide coatings by XPS.

Using these spectra, we estimated the O/Al and O/Zr stoichiometric ratios. The photoelectron spectra of Al₂O₃ (MS) oxides exhibited the binding energy peaks E(Al2p) – 74.4 eV and E(O1s) – 531.3 eV (figure 2). For ZrO₂ (MS) coatings, the binding energy peaks E(Zr3d) – 182.4 eV and E(O1s) – 530.2 eV were observed. Two peaks exist in the Zr3d XPS spectrum, showing an energy shift of 2.43 eV of the 3d level due to spin orbit coupling (figure 3).
The XPS spectra of the Ta$_2$O$_5$ (EB) films were also obtained. All spectra consisted of well-defined XPS lines of Ta 4f, 4d, 4p and 4s, O 1s. The Ta 4f doublets are typical for e-beam evaporated Ta$_2$O$_5$ and exhibit two peaks: Ta 4f7/2 at ~26.3-26.6 eV and Ta 4f5/2 with binding energy higher by 1.9 eV. The Ta 4f lines of the deposited films agree well with the Ta 4f doublet representative of the Ta-0 bond in Ta$_2$O$_5$. The O/Ta ratio estimated from the spectra is ~3 for all samples. Replacing the surface bonds with oxygen by either thermal or plasma treatment results in a shear in the more chemically stable hydrophilic surface region. However, the annealing process results in some additional impurities which were observed in the XPS spectra of e-beam evaporated Ta$_2$O$_5$ (C1s, Si2p, Na1s). The results show that the surface properties are strongly influenced by the preliminary treatment. The deposition and treatment conditions affect the properties of the coatings and the positive cell response [9].

The surface free energy (SFE), its polar and dispersion parts and fractional polarity estimations were made by the Wu method for two-liquids system and by Owens-Wendt-Rabel-Kaebler’s methods for the following liquid system: α-bromonaphthalene-formamide-ethylene glycol-diiodomethane-glycerol-water. The SFE varies practically independently from the surface roughness parameters in the range 2 – 400 nm. The SFE values were in the range 52 – 53 mN/m for Al$_2$O$_3$ (MS), 48 – 51 mN/m for ZrO$_2$ (MS) and 41 – 44 mN/m for Ta$_2$O$_5$ (EB) coatings. These results make it possible to distinguish between the influence of roughness and surface free energy effects on the cell/nanomaterial interactions.

We further studied the MSCs genes expression. An important component of the MSCs immune modulating activity is the enzyme indoleamine 2,3-dioxygenase (IDO) produced by these cells.
Figure 4 demonstrates the rise in the expression rate of the IDO gene in BMs after culturing on a glass/Al₂O₃ (MS) substrate in comparison with BMs cultured on a glass substrate after the 1st and the 2nd passage. Due to the increase of the cell content with MSCs markers during culturing on oxide nanocoating, a rise in the expression rate of IDO gene is to be expected, since in the culture of BM cells, namely MSCs, are IDO producers.

Conclusions
Our data demonstrate that the cells’ adhesive potential and phenotypical characteristics are different for the different oxide coatings deposited by magnetron sputtering and e-beam evaporation. The best results were obtained in the case of magnetron-sputtered oxide coatings with minimal roughness in the range 20 – 400 nm, intermediate values of the surface free energy as calculated by means of the Wu, Owens-Wendt-Rabel-Kaelble methods in the range 50 – 60 mN/m and the greater part of SFE polar components and fractional polarity. Changes were observed in the molecular-genetic apparatus of MSCs (IDO gene expression degree), together with an increase in the MSCs marker number on the oxides nano-structured surface. The results show the effect of the surface parameters modification on of the nano-materials interaction with mesenchymal stem cells and open prospects for a direct control of such parameters as adhesion, proliferation and differentiation of MSCs during their culturing.

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