Nanoscale surface modification of plastic substrates for advanced tissue engineering applications

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Abstract. Modified surface properties such as composition, nano roughness, wettability have effect on the most important processes at biomaterial interface. The research of stem cells (MSCs) adhesive potential, morphology, phenotypical characteristics on oxide coated and plastic substrate with different surface parameters was made. The oxide coatings deposition on plastic substrates shifts the surface properties at the more hydrophilic region and results in next positive cell/ biomaterial response in vitro tests. The MSCs marker number increases on the oxide nano structural surface of plastic substrates.

1. Introduction.
Recently, various stem cells, including mesenchymal stem cells (MSCs), have great potential in the tissue engineering area due to their biological capacity to differentiate into specific lineages and next advanced therapies applications [1-3]. Functional coatings deposition is an effective way of surface modification with direct control of stoichiometry, impurity elements, functional groups and surface charges. Coatings based on Ti, Al, Zr, Ta oxides exhibit unique properties: high inductivity, density, bio- and chemically inertness, which are very important for next implant and tissue engineering applications [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-submicronic level [6,7]. The separation of different factors influenced on cell/surface interaction such as nano topography, surface chemistry and surface free energy is of great interest for next tissue engineering applications.

2. Materials and Methods
At the present study the samples were plastic substrates (Petri dishes), uncoated and oxide coated (Al₂O₃, ZrO₂ (MS)) magnetron sputtered with different roughness parameters – 20, 200 and 400 nm. Al₂O₃ and ZrO₂ (MS) coatings deposition was performed in high vacuum pumping system with the base pressure about 10⁻³ Pa. The main process parameters were magnetron discharge power 1-8 kW, oxygen source power 1kW, deposition rate 8 µm/hour [8]. The deposition process in the case of fusible materials (plastic Petri dishes) has some difficulties - in the standard regimes the plastic Petri dishes melting. To optimize the regimes of deposition (the low temperature of samples and high rate
of coating deposition process) the additional research of magnetron plasma and inductive discharge parameters that influence on the temperature of substrate was made. The temperature of substrates depends both on the parameters of magnetron and induction discharges, substrate location and method of cooling. By means of thermocouple the temperature dependences and the parameters of discharge, distance to the substrate, temperature distribution at the deposition area were measured. The radial temperature distribution in the plane of substrate holder was presented (figure 1). Figure 2 demonstrates the temperature dependences for different regimes of magnetron sputtering oxide coatings deposition; magnetron power -5 kW, 2.5 kW and distance - 30 cm. The discharge power decrease results in significant temperature decreasing. The distance to the target shift from 25 cm to 30 cm also leads to temperature decreasing.

![Figure 1. The temperature radial distribution: magnetron power -5 kW, distance -25 cm](image1)

![Figure 2. The temperature dependences of different regimes of magnetron sputtering coating deposition process on plastic](image2)

The results allow to optimize the process of coatings deposition, to avoid the excessive heating of substrates and to select the most effective conditions for deposition process on plastic substrates.

The structure of Al$_2$O$_3$, ZrO$_2$ (MS) magnetron sputtered coatings were investigated by means of XPS and XRD methods. X-ray diffraction profiles of Al$_2$O$_3$ (MS) were observed by means of diffraction device “DRON-3” with filtered Cu-Ku radiation. X-ray photoelectron spectroscopy was carried out using ESCALAB MkII (VG Scientific) electron spectrometer at a base pressure in the analysis chamber of 5x10$^{-8}$ Pa (during the measurement 1x10$^{-6}$ Pa), using Al Kalpha X-ray source (excitation energy $h\nu=1486.6$ eV). The instrumental resolution measured as the full width at a half maximum (FWHM) of the Ag3d5/2, photoelectron peak is 1 eV [9].

The surface roughness was estimated by profilometer Hommel T-2000 measurements. The surface topography was investigated by means of Interferometric Microscope TalySurf CCI (Taylor Hobson) and AFM (Quesant Instrument Corporation, USA) methods.

The contact angles were measured by means of tensiometric method in Kruss K12 Tensiometer at temperature 20°C. The standard liquids with well-known values of surface tension, component of dispersion and polar interaction such as water, formamide, diiodo methane, ethylene glycol, α-bromo naphthalene and glycerol were used [9].

The others parameters such as surface free energy (SFE), its polar and dispersion components and fractional polarity were determined by means of Wu, Neumann-Kwok methods for plastic substrates.

Integral index of structural and functional state of cells reflecting their functional activity is adhesion. Therefore, experimental studies in vitro were started from the investigation of adhesive potential of bone marrow cells (BM), cultured on plastic uncoated and oxide coated substrates with different roughness parameters. MSCs phenotypical characteristics were determined by cytofluorimeter FACS Calibur with using fluorochromal monoclonal antibodies to CD73, CD106, CD44 structures. The CD105 fractures were extracted from cultivated MSCs by means of antimouse CD105 MultiSort Kit (PE) for next phenotype evaluation by cytofluorimetry.
3. Results and Discussion.

X-ray diffraction profiles of Al$_2$O$_3$ (MS), ZrO$_2$ (MS) as-deposited coatings demonstrate the amorphous nature, no peaks were observed. The tailored surface chemistry, stoichiometric composition and surface states of coatings are very important for next cell/biomaterial response. The structural analysis of Al$_2$O$_3$ (MS) and ZrO$_2$ (MS) oxide coatings by means of XPS method was presented. The photoelectron spectra of Al$_2$O$_3$ (MS) and ZrO$_2$ (MS) oxide demonstrate that oxide coatings have strong stoichiometric composition with binding energy peaks E(Al2p) – 74.4 eV, E(O1s) – 531.3 eV for Al$_2$O$_3$ (MS) films (figure 3 a, b). The binding energy peaks E(Zr3d) – 182.4 eV (figure 3 c), E(O1s) – 530.2 eV for ZrO$_2$ (MS) coatings were observed. At the Zr3d XPS spectra exist 2 peaks, demonstrated the energy shift 2.43 eV of 3d level due to spin orbit coupling [10].

The surface topography of investigated coatings deposited on the plastic substrates with different parameters of surface roughness was evaluated by means of Interferometric Microscope Talysurf CCI (Taylor Hobson) and AFM (Quesant Instrument Corporation, USA) methods. (figure 4).

The surface free energy SFE and its polar and dispersion parts, fractional polarity estimations were made by Wu method for two liquids system (ethylene glycol – water at temperature 20°C) and by Neumann-Kwok methods for plastic substrates, with various roughness parameters. The SFE was in the range 30-32 [mN/m] for plastic substrates and 43-45 [mN/m] for ZrO$_2$ and 54-55 for Al$_2$O$_3$ oxide coated on plastic substrates by Wu method.
The most hydrophobic nature, maximum values of advancing contact angles and minimum values of SFE for various roughness parameters in the range 20-400 nm possess plastic substrates. The hydrophobic properties of plastic substrates change in the more hydrophilic region and surface free energy increase for oxide coated plastic substrates (figure 5).

The influence of coatings roughness and composition parameters on BM adhesive potential was analyzed. The adhesive potential of BM cells was statistically and significantly different depending on substrate material properties. The best results were obtained on substrate surfaces with minimum roughness parameters in the range 20-400 nm.

The study of the expression of MSCs genes was made. An important component of immune modulating activity of MSCs is enzyme indoleamine 2,3-dioxygenase (IDO) which is produced by cells. The rise in the expression rate of IDO gene in BM after culturing on plastic/Al$_2$O$_3$ (MS) substrate in comparison with BM cultured on plastic substrate after the 1st and the 2nd passage was observed (figure 6).

**Figure 5.** The surface free energy SFE and its polar and dispersion parts [mN/m] 1-3 – plastic with roughness 20, 200, 400 nm, 4-6 – plastic 20, 200, 400 nm with oxide coatings

**Figure 6.** Expression rate of IDO gene in cultured BM on various substrates. 1 – Ladder (molecular marker of fragments’ length); 2 – MSCs (plastic+Al$_2$O$_3$); 3 - MSCs (glass+Al$_2$O$_3$); 4 – Negative control; 2,3 – Upper line – IDO transcripts (target gene), lower – beta-actin (house keeping gene); ido gene transcripts located in the zone of 200-350 b.p.

Due to the increase of the cell content with MSCs markers during culturing on oxide nano structural surface, rise in the expression rate of IDO gene is logic since in the culture of BM cells, namely MSCs are IDO producers.
The rise in the mentioned transcripts of the cells with MSCs markers during BM culturing on oxide nano structural surface was demonstrated both for plastic and glass substrates.

4. Conclusions.
The best MSCs adhesive potential and phenotypical characteristics were obtained in the case of magnetron sputtered oxide $\mathrm{Al}_2\mathrm{O}_3$ (MS), $\mathrm{ZrO}_2$ (MS) coatings with minimum parameters of roughness in the range 20-400 nm and intermediate values of surface free energy in the range 45-55 mN/m, the greater part of SFE polar components and fractional polarity calculated by means of Wu, Neumann-Kwok methods for plastic substrates with various roughness parameters. The changes at molecular-genetic apparatus MSCs (IDO gene expression degree) and MSCs marker number increasing on the oxide nano structural surface of plastic substrates were observed. The results show the significant effect of surface parameters modification on the regularities and mechanisms of nano materials interaction with mesenchymal stem cells (MSCs) in vitro tests.

Acknowledgment
The study was supported by the Program for Cooperation between Bulgarian Academy of Sciences and the National Ukrainian Academy of Sciences.

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