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The effect of surface treatment of ceramic oxide coatings deposited by magnetron sputtering method on the adhesive and proliferative activity of mesenchymal stem cells

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ABSTRACT

During the last years the interest to mesenchymal stem cells (MSCs) has significantly increased due to diverse clinical application of MSCs. As a result, it appears necessary to develop methods to increase the number of MSCs by culturing bone marrow (BM) cells *in vitro*, mainly to achieve consistent control of cell adhesion and proliferation processes at the biomaterial interface. In this study, we have analyzed the effect of surface treatment by argon ions bombardment and the electron beam irradiation on the structural and surface properties of the ceramic Al_2O_3 coatings by TEM, SEM and AFM methods. The coatings were deposited by reactive magnetron sputtering and surface characteristics were correlated with cultured MSCs adhesion. Finally, it could be shown that after electron beam irradiation Al_2O_3 coatings increased the adhesive and proliferative potential of MSCs compared to as-deposited oxide coatings.

1. Introduction

Biomaterials have different mechanisms of biomolecules and cells adsorption. Implants used in traumatology and orthopedics for osteosynthesis commonly benefit from adsorption of proteins on their surface (collagen or fibrinogen facilitates integration of implant with the bone tissue). On the other hand, in case of designing of artificial heart valves, blood vessels, catheters, or endoscopes, the proteins adhesion can lead to thrombosis and therefore should be minimized [1,2]. The mechanisms of both processes are dependent on a number of factors, such as surface characteristics of biomaterials and biological properties of the surrounding environment (level of pH, electrolytic reactions, and composition of proteins) [3,4]. These factors play a key role in cell adhesion: platelets, red blood cells, osteoblasts, and microorganisms that affect the working quality of the various implants and devices. In turn, the adhesion of cells and proteins depends on biomaterial surface parameters, such as surface topography, roughness, free energy (SFE), and surface charge states [5–9].

The application of nanostructured coatings can reduce the free enthalpy of adsorption of biological macromolecules. By adsorption of ions of the extracellular fluid, new surface states can be formed at the biomaterial surface that change surface electronic states, particularly in the case of non-stoichiometric compounds.

During the last years, a new branch of medicine based on the use of nanocomposite coatings is rapidly developing. It promotes positive biological processes in a living organism. Medical products coated with ceramic coatings form normal biopotentials in damaged areas of tissue and organs, that prevent necrosis, tissue atrophy, and significantly accelerate the healing process and post-surgical rehabilitation [10–13]. Moreover, during surface treatment of artificial vessels, blood coagulability can be kept under control and the change of surface parameters would allow reducing the likelihood of thrombosis [14].

Today, interest in mesenchymal stem cells (MSCs) has significantly increased due to their high plasticity, *i.e.* their ability to differentiate into both mesenchymal tissues and derivatives of other germinal layers. Due to the ability of self-renewal, multilinear differentiation and immunologic properties of MSCs recently have gained attention of the experts in the fields of cell biology, experimental and clinical medicine [15–17]. Immune modulating potential and immune suppressive activity of these cells are the basis of clinical trials in therapy of different diseases [18,19]. The clinical application of MSCs requires improvement of

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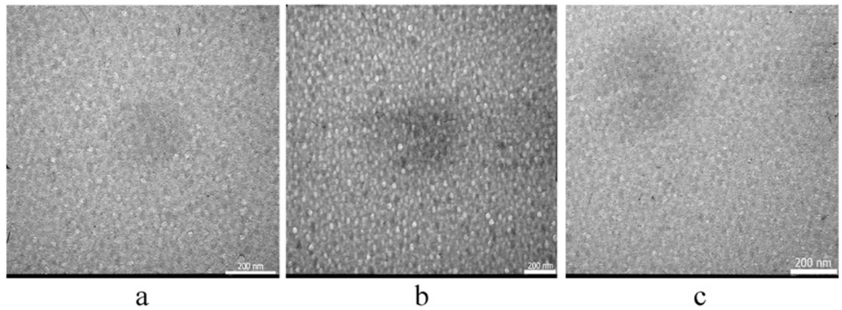

Fig. 1. Brightfield TEM images of as-deposited oxide ceramic coatings: a) Al_2O_3 coatings, b) Al_2O_3 coatings deposited with simultaneous bombardment by argon ions, c) Al_2O_3 coatings after electron beam irradiation post-treatment.

traditional culturing methods to preserve morpho-functional properties of cells and produce required quantities for medical use [20].

Alumina has been used occasionally as an implanted inorganic scaffold material in tissue engineering due to its biocompatibility and mechanical properties. The reports of Refs. [21,22] demonstrate the ability of nanostructural alumina surfaces to promote cell adhesion, proliferation and differentiation for further advanced tissue engineering applications.

Novel ceramic coatings are applied in different industrial areas thanks to their high hardness and wear resistance properties [23].Various modern techniques are widely used for functional coatings deposition such as electrochemical oxidation, sol–gel, ion-beam assisted deposition (IBAD) and others [24–26]. Magnetron sputtering (MS) is one of the most popular methods of ceramic coatings deposition due to the possibility to precisely control the uniformity and stoichiometric composition of produced coatings [27–31].

Numerous studies have focused on the cell/biomaterial response of nanostructured surfaces. However, there is insufficient information about the surface treatment effects on MSCs cellular response. The aim of the present study is to analyze the effect of surface treatment by argon ion bombardment and electron beam irradiation on the structural properties of ceramic Al_2O_3 coatings, deposited by reactive magnetron sputtering. Furthermore, a correlation between the surface characteristics and the adhesion of cultured cells was identified.

2. Materials and methods

The substrates for coating deposition were glass Petri dishes of 3 cm diameter (five samples with investigated coatings were used for one biological test). Other coatings were simultaneously deposited on NaCl substrates for TEM, and standard silicon and glass substrates for SEM analyses. The substrates were ultrasonically cleaned in acetone, 96% ethanol, distilled water, and subsequently dried in a drying oven.

Alumina coatings were deposited in a high vacuum pumping system with a base pressure of about 10^{-2} Pa by ion source-assisted magnetron sputtering. A pure aluminum target was used. The distance to the substrate was about 30 cm. Power to the sputtered cathode was applied using 10 kW DC power supply operated either in current or voltage regulation mode. The magnetron system was equipped by a coil of the magnetic field, permanent magnet, RF generator and inductive coil. Argon was used as the sputtering gas. Oxygen for the reactive deposition was delivered through an ICP plasma source. Flows for both argon and oxygen were regulated using mass flow controllers operated by a two-channel process control unit. The magnetron discharge power was between 1 and 4 kW, the power of the activated oxygen source was up to 1 kW, and the coating deposition rate was 8 μm/h [31,32]. The sputtering process was performed in the regimes far from the target poisoning areas to obtain ceramic coatings with highly stoichiometric composition. In addition, such deposition conditions allow avoiding micro-arcing and micro-drop formation.

For other samples, the deposition process was carried out with simultaneous bombardment of the growing film by argon ions using an ion source. The ion source parameters were as follows: ion acceleration voltage — 2.5 kV, current ion source — 30 mA. A primary electron beam was created by electron gun type UL-119. The energy of reflected electrons was 20 kV, the current density on the surface of sample — 14 mA/cm^2, the irradiation time — 1500 s.

The structure parameters of as-deposited and post-treated coatings were investigated by transmission electron microscopy (TEM) using JEOL JEM 2100 equipment. The surface topography and roughness parameters of the oxide ceramic coatings were evaluated by scanning electron microscopy SEM (QUANTA 600 FEG) and atomic force microscopy AFM (NTEGRA-AURA). The statistical processing of the AFM images was performed using the software package "Image Analysis P9" (NT-MDT). The coating thickness and cross-section structure were obtained from a computer-controlled SEM (Nova NanoSEM, FEI) cross-section measurements. The dielectric properties of oxide coatings were characterized by a computer-controlled LCR-meter BR-2876. Frequencies were chosen in the range from 100 Hz to 1 MHz with an oscillation voltage of 1 V. Capacity, dielectric constant, and parameters of dielectric loss tangent depending on the frequency of electromagnetic field for oxide ceramic coatings deposited in the different sputtering

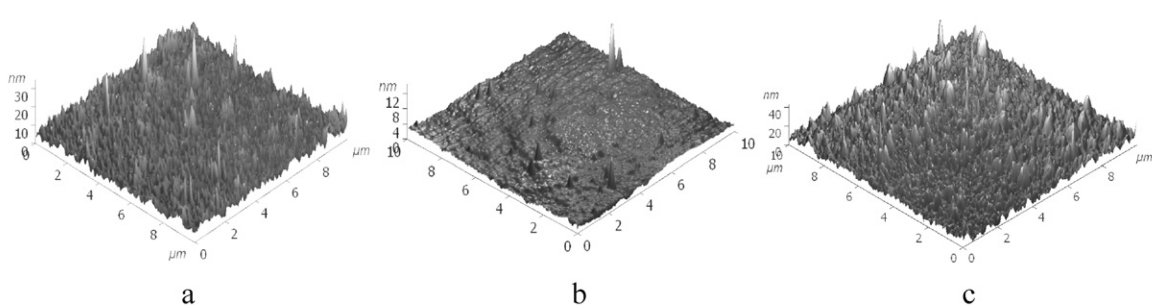

Fig. 2. AFM images of as-deposited oxide ceramic coatings: a) Al_2O_3 coatings, b) Al_2O_3 coatings deposited with simultaneous bombardment by argon ions, c) Al_2O_3 coatings after electron beam irradiation post-treatment.