INFLUENCE OF BIOCOMPATIBLE COATINGS ON MAGNESIUM ON THE FUNCTIONAL STATE OF MESENCHYMAL STEM CELLS

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Abstract

This article reports the bioactive resorptive coatings, formed on magnesium alloy substrates, using the method of plasma electrolytic oxidation (PEO), and their cytocompatibility with bone marrow derived mesenchymal stem cells (MSC). Were obtained the protective coatings containing hydroxyapatite with a ratio Ca/P = 1.6 close to the bone tissue index (1.67) on the magnesium alloy MA8 in the electrolyte solution containing calcium glycerophosphate and sodium fluoride in the bipolar regime of PEO. These coatings have a developed porous surface and significantly reduce the rate of corrosion of the magnesium alloy, which makes it possible to consider them as perspective for bioresorbable medical implants. Such bioactive coatings that modify the surface of magnesium provide a porous surface that promotes the strong attachment of MSCs.

Key words: *magnesium alloys, bioactive resorptive coatings, biodegradation, mesenchymal stem cell, osteogenesis*

1. INTRODUCTION

In modern orthopedics and traumatology, for stable-functional osteosynthesis in bone fractures, metal implants of titanium are widely used. Nevertheless, the chemical composition of metals changes under the influence of the environment, and time spent in the body despite of sufficient mechanical strength of structures (Motarano et al., 2011, Kumar et al., 2015, Schlundt et al., 2015). The presence of toxic ions in the surrounding tissues can cause allergic reactions, which come out as an aseptic inflammation (Pierre et al., 2010, Li et al., 2017). Moreover, a re-operative intervention for removing the metal structure after consolidation of the fracture is negative. One of the materials that we can perspectively use as a bio-degradable implant is magnesium. It is shown that, Mg has a density similar to cortical bone and has nontoxic and biocompatible properties (Amini et al., 2011, Kraus et al., 2012, Bondarenko et al., 2014). Magnesium-based implants are completely resorbed while maintaining sufficient mechanical integrity of the bone tissue during osteogenesis (Charyeva et al., 2014). So was show the pharmacological actions of magnesium ions, which on the one hand promote accelerated regeneration of bone tissue (He et al., 2016), and, on the other hand, suppress inflammation (Amini et al., 2011).

One of the properties of magnesium is its ability to resorption, which limits its use as an implant with long-term consolidation of bone tissue. There are various ways to increase the mechanical strength of chemically pure magnesium, for example, anodizing, alloying (fusing) metals (Li et al., 2008, Amini et al., 2011). This way, elements such as Ca, Zn, Nd and Y are injected into magnesium alloys in different concentrations. The obtained alloys can significantly increase the resistance of polarization and reduce the rate of dissolution of the material in a physiological environment. However, the biodegradation of pure magnesium and alloys is a complex process and its rate depends both on the composition and the properties of the impurities (Hofstetter et al., 2015a, Hofstetter et al., 2015b; Yang et al., 2015). Accelerated generation of corrosion products of the Mg implant, such as H_2, OH^- and Mg^{2+} ions, can lead to the formation of a gas cavity in surrounding tissues with subsequent inflammation.

The best way to achieve certain corrosion resistance is to use the chemical modification of the magnesium alloy surface by phosphates, calcium phosphates, bioglass or biodegradable polymers using anodization, plasma electrolytic oxidation or sol-gel synthesis technologies (Wong et al., 2010, Gao et al., 2011, Hornberger et al., 2012). Moreover, coatings on magnesium alloys should have certain properties, such as, biocompatible with the environment of the body, bioactive to the restoration of damaged tissues or organs, and the possibility of gradual resorption. The formation of calcium phosphate coating (Ca-P), including hydroxyapatite-containing, on magnesium and its alloys is mainly carried out by the method of plasma electrolytic oxidation (PEO) (Srinivasan et al., 2010). The presence of calcium ions and phosphate ions in the simulated body fluid (SBF) provides the formation of hydroxyapatite on the surface of the magnesium implant, which plays an important role in the processes of osteoinduction. To assess the rate of resorption of magnesium alloys, model physiological fluids of SBF, Hank and Ringer-Lock, containing chloride ions are used (Gray-Munro et al., 2013, Cha et al., 2013). However, the effect of such surface factors on cellular adhesion, growth properties, and cell viability was rarely studied for both magnesium alloys and coatings formed on it.

For the purpose of bone tissue regeneration, multipotent mesenchymal stromal cells (MSC) of the bone marrow are most active in cellular technologies (Kimbrel et al., 2014, Meirelles & Nardi, 2014). Common properties of MSC are fibroblast-like morphology, high proliferative potential, ability to adhesion, and easily induced differentiation in osteogenic, chondrogenic and adipogenic directions (Polymeri et al., 2016). Specific markers of differentiation (CD73, CD90, CD105, CD166) are expressed on the surface of MSC and their functional feature is the ability to synthesize angiogenic cytokines (Panaroni et al., 2014, Ullah et al., 2015). It was shown that bone marrow MSCs have a higher osteogenic potential than MSC of adipose tissue (Deng et al., 2015). In osteoid differentiation, we notice constant expression at all stages of the hierarchical ladder of the early genes of type I collagen and the transcription of nuclear binding factor a1 Cbfa-1 (core binding factor a1), the structural element of a number of Osx (Osterix, osterix). Typically, heterogeneous population with an evident osteogenic potential indicated by small amount of cells is represented in colony forming (CFU-f) culture of bone marrow MSCs islets (Tamama & Kerpedjieva, 2012). With further cultivation in an environment that induces osteogenesis, osteoid differentiation cells are detected by the formation of mineralized islets of bone cell development (Deshpande et al., 2013, Grayson et al., 2015). In the formation of such islands, not only osteoprogenic cells take part, but also other adhesive and non-adhesive bone marrow cells which create the necessary fund of supporting cells. Despite the presence of a multitude of cytokines active against bone cells, in the early phases of osteopoiesis, in the absence of osteopoietic additional bone marrow cells, precursor cells of osteocytes die. It is suggested that osteopoetic additional cells form a soluble regulator that affects the proliferation and differentiation of the population of osteoprogenic cells.

The purpose of this study is to study the morphofunctional state of MSC of the bone marrow upon contact with magnesium alloy (MA8) and anticorrosive bioactive resorptive coatings, formed on it, using the method of plasma electrolytic oxidation.

2. MATERIALS AND METHODS

2.1. Objects of research

The experiments were carried out with the following samples: uncoated - magnesium alloy MA8 (Mg 1); with calcium phosphate coating Ca/P = 1:1 on MA8, formed by the PEO method (Mg 2); and with a pre-formed PEO layer on MA8 containing particles of ultradisperse polytetrafluoroethylene (UPTFE, Mg 3).

The magnesium alloy MA8 contained 1.5-2.5 wt. % Mn, 0.15-0.35 wt. % Ce, the the rest was Mg. For the application of coatings, 15 mm × 20 mm × 2 mm plates were used, pre-machined to a roughness level of Ra = 0.12 μm. After grinding, the samples were washed in distilled water, and the surface was degreased with alcohol. Plasma electrolytic oxidation (PEO) was carried out in an electrolyte containing 25 g / l of calcium glycerophosphate, (C3H7O6P) Ca·2H2O, and 5 g / l of sodium fluoride, NaF, at a current density of 0.67 A / cm2 for 300 s, the voltage at the anode reached 540 B. the pH of

the electrolyte was adjusted to 10.9-11.3 by adding a 20% NaOH fluid. As a current source, a reversible thyristor unit was used. The frequency of the polarizing pulses was 300 Hz. The control of electrical parameters was carried out by an automated control and monitoring system, coupled with a computer, equipped with appropriate software.

To seal the pores of the PEO layer (creating a composite polymer-containing coating), we used UPTFE with averaged fractional composition (trademark Forum®), obtained by gasdynamic thermo-dispersing method (Kuroda & Okido, 2012). Additional processing of the samples with the preformed PEO layer was carried out by short-term (10-15 s) immersion in a slurry containing 0.2-0.6 μm particles of UPTFE in isopropyl alcohol, with the addition of the OP-10 wetting agent, with the following components content (g/l) : UPTFE - 100-150; OP-10 - 6.0-8.0; isopropyl alcohol - the rest. After immersion and complete evaporation of the isopropyl alcohol from the surface of the sample, the polymer layer was fixed onto the PEO coating by thermal treatment at 310-380 ° C for 3 minutes.

The samples were sterilized before use in a dry-fire cabinet (Thermo Electron, Germany) at 180 °C for 15 minutes, which corresponded to the rules for sterilization of medical devices, monitoring the surface properties of the samples during the process.

2.2. Animals and cellular cultures

Study approval from the local Ethical Committee of the Pacific State Medical University (Vladivostok, Russia) was received under No. 2015-0102. Male guinea pigs were purchased from a laboratory of the Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry (Pushchino, RU). Guinea pigs in the age range of 8–12 month (250–280 gr) were used in all experiments.

The primary culture of undifferentiated MSC was obtained by the method of D. Foudah with co-authors from the femur of animals by washing the medullary canal with RPMI-1640 medium, containing 25 units of heparin (Biolot, Russia). Bone marrow was homogenized on a dissociator (Milteni Biotec, Germany), washed three times with DMEM-LG medium by centrifugation and transferred to enriched environment for cultivation.

As the main medium for proliferation of the MSC cells we used the DMEM-LG medium containing glucose 1 g/L, 2 mM glutamine (LONZA, BioWhittaker ™, Belgium), 1 mM sodium pyruvate, 12.5 mM HEPES-buffer (Sigma, USA), 100 units / ml of penicillin and 100 μg / ml of streptomycin (Biolot, Russia), 10% fetal bovine serum (Hyclone-Defined, USA). The cultivation was carried out at 37 ° C in a CO2 incubator (Thermo Scientific, USA), the medium was replaced every 3-4 days with the evaluation of the cell morphology. When the cells reached 90% confluency, the cells were passaged in a ratio of 1:3. During this procedures we used a 20 mM phosphate buffer and a solution of 0.25% trypsin / 0.05% EDTA (Biomedicals, USA).

After a 3-fold passage, osteogenic differentiation of MSCs was performed by culturing the cells in DMEM-LG or DMEM-advanced medium (LONZA, BioWhittaker ™, Belgium) containing 10-8 M dexamethasone (Sigma, USA); 10 mM beta-glycerol-2-phosphate and 0.2 mM 2-phospho-L-ascorbic acid (Fluka, Germany) with a density of 2500-5000 cells / cm2 in vials with samples and coverslips. The cells were removed from the samples after incubation (3, 5, 7, 14, 21, 28 and 42 days) by inclusion into the vials a Vershen solution, containing 0.25% trypsin (PanEco, Russia).

2.3. Methods for determining the functional state of cells

To study the adhesion of cells to the test samples, non-adherent cells were washed with Hanks solution (200 μl, Lonza, USA) and counted using a MACSQuant flow cytometer (Miltenyi Biotec, Germany). The cytotoxic properties of the samples were determined by conducting a test for the cells incorporation of methylthiazolyltetrazolium (MTT). For this purpose, a fluid containing 2 mg / ml of MTT (3- [4,5-dimethylthiazolyl-2] -2,5-diphenyl tetrazolium bromide, Sigma-Aldrich, USA) was added to the cell monolayer on the basis of phosphate buffer (pH 7.2) with 0.4% MnCl2 and was incubated at 37 ° C for 30 min. Cells with included dicazan granules were destroyed by applying 100 μl of isopropyl alcohol acidified with 0.04 M HCl for 20 min. with incubation on a thermosweaker ("Bio-Rad", USA) at 37 ° C and the substrate optical density was measured on an immunological analyzer "Multiskan FC "(Thermo Fisher Scientific, Finland) at a wavelength of 540 nm.

Measurement of inclusion by MTT cells was carried out on the same number of samples, five indices at one time point. The results of spectrophotometric analysis were shown in a stimulation index (T,%). The index was calculated as the ratio of the difference between the average of optical density of fluids, containing the reaction products of the cells, contacted with the samples and with the glass (control), to the average of optical density of the fluid for the cells, after contact with the glass, the control was taken as 0.

Cell viability was determined by the penetrance of the membranes for acridine orange (AO, 250 ng / µl, Sigma-Aldrich, USA) based on 0.1 M citrate buffer (pH 6.0) including 1 mM EDTA-Na, 0.15M NaCl, 0.2M NaPO4 and 1 mg / ml albumin from bovine serum (Sigma-Aldrich, USA). After 15 minutes, the stained cells were suspended and analyzed on a flow cytometer with identification of a small angular light scattering (FSC) that characterizes a cell size, lateral light scattering (SSC), which characterizes cytoplasmic cell features. Cell fluorescence was also registered at 525 nm and 585 nm emission wavelengths with the integration of the compensation coefficient analysis into the program (Plemel et al., 2017). Measurement of AO activation by the cells was carried out on the same number of samples, five indices per one time point.

To study the ultrastructure by means of a scanning electron microscope, a monolayer of cells was treated with a complex Ito fixator. Postfixation was carried out in a 1% fluid of osmium tetroxide, dehydration - in acetone fluids of ascending concentration, followed by spraying on the surface of carbon cells with thermal evaporation in a vacuum. Images of the samples were obtained with an electron microscope Ultra 55 (Carl Zeiss, Germany) at an accelerating voltage of 0.8-1 kV.

2.4. Statistical analysis

The description of a sampling was made by calculating the median (Me) and by interquartile range which corresponded to 25 and 75 percentile (C25 and C75). The reliability of the differences between the indices of independent samples was estimated from the nonparametric Mann-Whitney criterion. Two way analysis of variance (ANOVA) with cells on the glass and cell contact with patterns as independent parameters was used to evaluate statistical significance at $p < 0.05$. Post hoc analysis was performed to determine multiple comparisons using the Tukey method (Origin 9.1 software, OriginLab).

3. RESULTS AND DISCUSSION

The electrolyte composition for PEO was developed to form the coating having simultaneously bioactive and anticorrosion properties. Calcium glycerophosphate comprises the calcium salt of phosphoglycolic acid. Glycerophosphate-ion $(C_3H_7O_6P)^{2-}$ contains both organic (radicals of triatomic alcohol – glycerol, $C_3H_4(OH)_3$) and inorganic (anions of orthophosphoric acid, H_3PO_4) parts. Upon entering the plasma discharge, organic components, due to high combustion heat, increase the temperature of the discharge channel and, therefore, promote electrolyte intensive thermolysis and hydroxyapatite synthesis in the coating composition in accordance with the reaction:$10Ca^{2+} + 6PO_4^{3-} + 2OH^- \rightarrow Ca_{10}(PO_4)_6(OH)_2$.

As a result of magnesium alloy treatment by the PEO-method, a coating consisting, according to the X-ray diffraction analysis, of magnesium oxide and calcium phosphate, including hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) was formed. The coating internal layers adjacent to metal contain magnesium fluoride. Formation of the chemically stable MgF_2 is provided by sodium fluoride purposefully introduced to the electrolyte composition.

The characteristic feature of the coatings formed using the PEO-method consists in the presence of an external porous sublayer reducing the coating corrosion resistance at large. In order to seal the available pores, impregnation of the sample with the PEO-layer in UPTFE alcohol suspension and subsequent thermal treatment were used. The used method allowed sealing pores of the base PEO-layer by a chemically stable inert polymer.

Figure 1 shows the dependence of the volume of the evolved hydrogen ($V[H_2]$) on the time of immersion in the SBF-solution for the studied samples with different types of surface layers. Hydrogen formation occurs due to magnesium corrosion, which can be described for neutral and alkali media by the equation: $Mg + 2H_2O \rightarrow Mg^{2+} + 2OH^- + H_2$.

As seen from the data, in the case of magnesium alloy without surface treatment, the volume of the evolved H_2 was 30 ml within 28 days of the sample immersed in the SBF-solution. The PEO-layer increases the corrosion resistance of the Mg-alloy in 3 times. For the sample Mg 2 (MA8+PEO), $V[H_2]$ was equal to 10 ml. The best protective properties characterize the sample with the compositepolymer-containing coating. No hydrogen evolution was observed within 28 days of the sample immersed in the SBF-solution. The samples appearances after exposure to the SBF-solution are shown in figure 2.

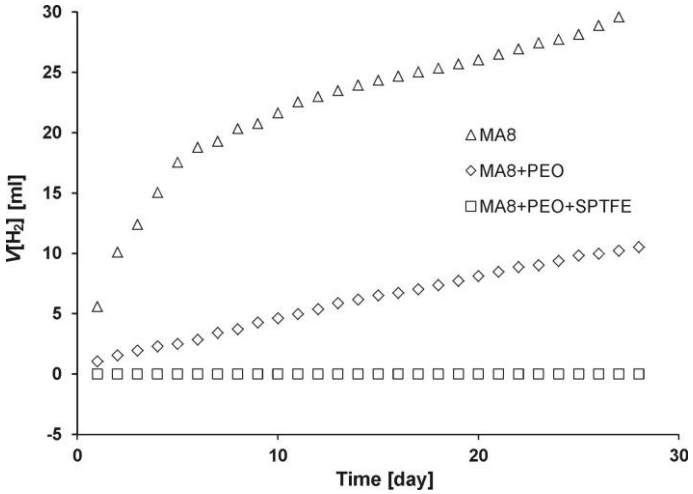

Fig. 1. Dependence of the volume of the evolved H_2 on the immersion time of studied samples of magnesium alloy MA8 in the SBF-solution.

Deposition of the composite polymer-containing coating on the magnesium implant allows substantial reduction of the alloy corrosion intensity (without its complete inhibition) through providing low corrosion activity of surface layers within the period of recovery of the bone integrity, whose duration is, as a rule, 12–14 weeks. As it was established in studies of biological properties of magnesium alloys, the most active cell adhesion was observed for the composite polymer-containing coating (Fig. 3A). Upon 30-minute contact with the above coating, 11.2±0.5 % of cells was adhered to the sample, whereas in cases of magnesium alloy without coating and with PEO-coating containing hydroxyapatite the cells quantity was smaller (6.6±0.5 % and 7.75±0.05 %, respectively).

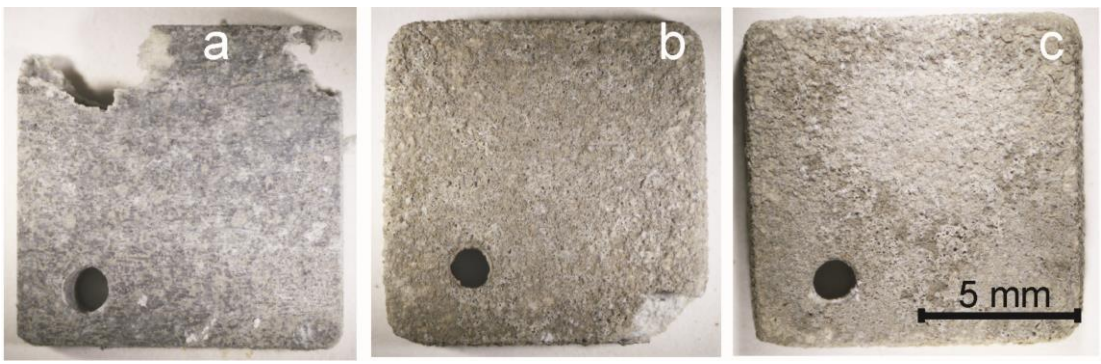

Fig. 2. Samples appearance after immersion in the SBF-solution: a – Mg1 (MA8); b – Mg2 (MA8+PEO); c – Mg 3 (MA8+PEO+ UPTFE).

A large quantity of adhered cells of the round shape with numerous valve- and thread-like pseudopodia around their perimeter at 30 min of contact with magnesium alloy Mg 1 were observed (Fig. 3B). In the course of time (9–24 h), the number of cells on Mg 1 significantly decreased and they were increased in size because of flattening of their shapes. In intercellular environment the abundance of excreted protein substances was observed. The architectonic of cellular surface on coatings magnesium alloy was differed. On the magnesium alloys with coating formed by Plasma Electrolytic Oxidation and calcium phosphate Mg 2 the cells less spread over the surface predominantly with fibril-like pseudopodia were observed (Fig. 3C). The expressed cell stimulation was noted in the case if they were in contact with composite polymer-containing coating Mg 3 (Fig. 3D). It was indicated the emergence of numerous round-shape folds on the cell surface and also the groups of cells in close contact mediated by numerous elongated pseudopodia. In the same time period, on Mg 2, the cells in the activated state with folded surface were revealed. One also observed some specific multicellular formations, in which morphologically separated cell were linked to each other through cytoplasmic bridges. Similar formations were also revealed at contacts with the composite polymer-containing coating Mg 3, but at an earlier stage – within 3 hours. The latter indicates to the cells chemotactic activity.

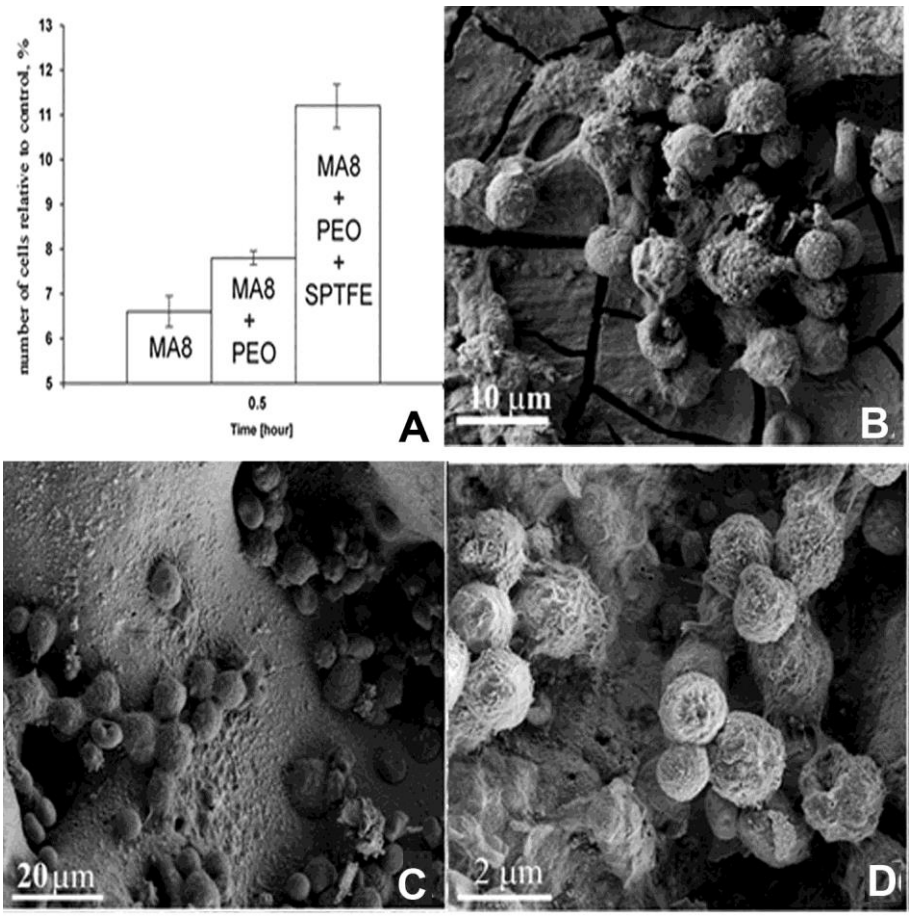

Fig. 3. Adhesion of cells on the surface of the test samples. A - histogram of the distribution of the number of cells that do not adhere to the surface after 45 minutes (1), 3 (2), and 5 hours incubation. SEM images of MCK: B – 3 days of contact with magnesium alloy Mg 1; C - contact with coating formed by Plasma Electrolytic Oxidation and calcium phosphate Mg 2 (3 days); D – contact with composite polymer-containing coating Mg 3 (3 days).

Under certain conditions, the ability of NADP-H-dependent oxidoreductase enzymes, in particular succinate dehydrogenases (SDG), to restore MTT to insoluble formazan granules, accompanies the

functional activity of the cells. This property of enzymes is used to determine the cytotoxicity of biologically active substances against cells in the "in vitro" model. The results of studying the effect of magnesium alloy with coatings and without them on MTT incorporation by cells were expressed as an index (T,%), control (C3) was taken as 0 (Fig. 4). When evaluating the cytotoxic effect of the studied coatings, an ambiguous cell reaction was observed. Thus, in the initial period of observation (12 h) there was an increase in SDG activity indices, which indicated the absence of cytotoxic effect of the studied samples (Fig. 4). A significant increase in the intracellular content of the formazan granules during this period reflected an increase in metabolic activity associated with the adhesion of cells to the surface of the samples. Moreover, the maximum values were found for the cells ($p < 0.05$) that contacted the PEO coating, containing the UPTFE. In this case, the SDG values after 5 hours of contact were: for the magnesium alloy (Mg 1) - 4.9 ± 0.45; for calcium phosphate PEO coating (Mg 2) - 23.3 ± 1.9 and PEO coatings with UPTFE (Mg 3) - 52.2 ± 5.68%. At the end of the observation period (30 days), a decrease in SDG activity was detected and a negative value of the index showed the presence of cytotoxic impact of magnesium and coatings applied on it.

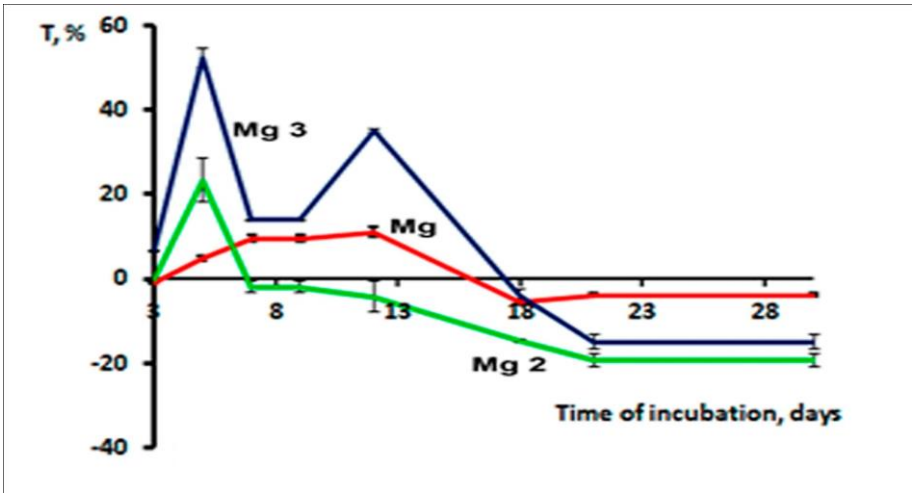

Fig. 4. Succinate Dehydrogenase Activity in MSC, Spectrophotometry Data Inclusion by Cells of Methylthiazolyltetrazolium. Mg 1 magnesium alloy; Mg 2 calcium phosphate PEO coating; Mg 3 PEO coating with UPTFE

Fluorescent vital dye acridine orange (AO) is used to study the number and structure of nucleic acids, which reflects the functional state of cells, connected with their viability and proliferation. When the wavelength of the ultraviolet spectrum (495 nm) is applied, the monomers of the dye bound to RNA and single-stranded DNA are capable of emission of the wavelength of the orange spectrum of 580 nm, when the quantum yield of the fluorescence of monomers of AO, sorbed by double-stranded DNA structures, lies in the area of a bright green spectrum of 520 nm. It was found out that with the AO distribution, the MSC cores fluoresce in the green area of the spectrum with a maximum of 520 nm, with the most intense fluorescence observed in the nucleolus, and the cytoplasm is characterized by a weak fluorescence.

The detection of fluorescence of MSC stained with AO, using a two-parameter analysis of the green spectrum against orange, allowed us to characterize the functional state of the cells after the exposure of the samples (Fig. 5). A low percentage of cells that fluoresce only in the orange spectrum, reflecting their death with increased permeability of the membranes, was noted in the population of uninduced and induced MSCs after contact with glass (controls C1, C2, C3) and PEO coating, containing UPTFE on Mg (Mg2). Upon contact with magnesium without (Mg) and with calcium phosphate PEO coating (Mg1), the percentage of cell necrosis was significantly higher ($p < 0.05$). Also, the percentage of fluorescent cells at two wavelengths was different, which reflects the dependence of the functional state on the sample with which they were in contact. This way, for non-induced (C1) and induced

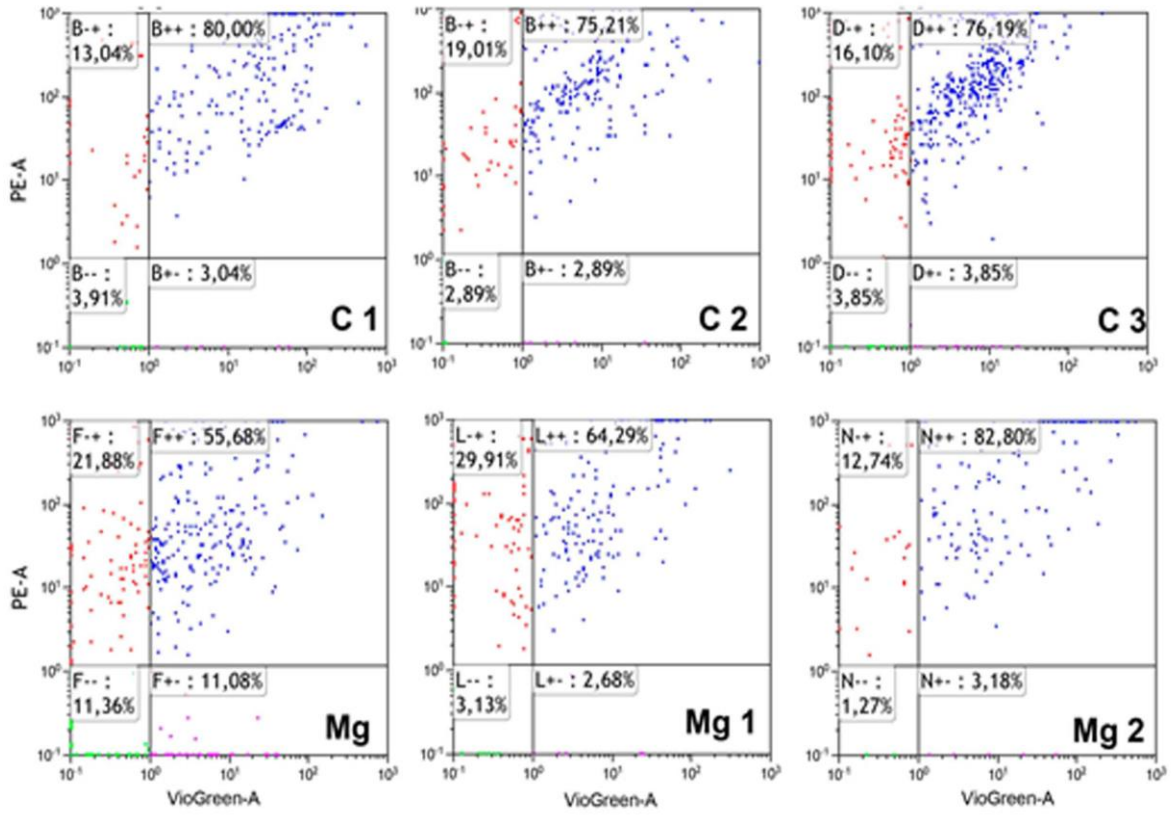

Fig. 5. Histograms of the distribution of cells by the number, size and incorporation of acridine orange dye. C - MSC without an inducer of differentiation after contact with plastic; C 1 - cells without differentiation inducer after contact with plastic; C 2 - MSC with inducer of differentiation after contact with collagen coating on plastic; Mg 1 - MSC after contact with the magnesium alloy; Mg 2 - MSC after contact with calcium-phosphate PEO coating; Mg 3 - MSC after contact with PEO coating with UPTFE. Flow cytometry, fluorescence excitation by an argon laser (495 nm), registration was carried out in two spectra: green (500-560 nm) and red wavelengths (590-660 nm).

MSCs (C2, C3) that contacted glass, the percentage of such cells did not differ significantly from 75.21 ± 7.3% to 80 ± 6.8%. Upon contact with magnesium (Mg) and calcium phosphate PEO coating (Mg1), the percentage of cells fluorescing in the two spectra was significantly lower and was in the range from 55.68 to 64.3%, with a high value for MSC, after the impact of PEO coatings with UPTFE (82.8 ± 8.3%). These spectra characterize the accumulation of AO in both monomeric and aggregated forms.

We already know, that the MSC of the bone marrow is heterogeneous in terms of morphological features and the potential for their ability to divide. According to the literature, small cells with a size of up to 10 μm have the greatest potential for proliferation. As they transform into osteocytes, which have dimensions on a short axis of 10 μm and over a length of 20 μm, these cells lose the ability to proliferate. To determine the effect of the samples on the differentiation of MSCs to osteocytes, we used the ability of a flow cytometer to separate the cells according to the sizes using a lateral scattering scale (FSF, Fig. 6A). It was found out that, the percentage of cells larger than 10 μm varied depending on the sample with which they contacted (Fig. 6). So, the greatest amount was observed for cells after their contact with calcium phosphate coating on magnesium and was 29.13 ± 1.8 after 21 days of cultivation (Fig. 6D). While in contact with the glass surface without and with collagen, magnesium and complex PEO coating with UPTFE at the same incubation times, the percentage of cells larger

than 10 μm was 20.04 ± 1.7; 21.7 ± 2.1; 15.23 ± 1.4 and 25.47 ± 2.5%, respectively. In general, these data indicates the osteoinductive properties of the tested coatings on magnesium.

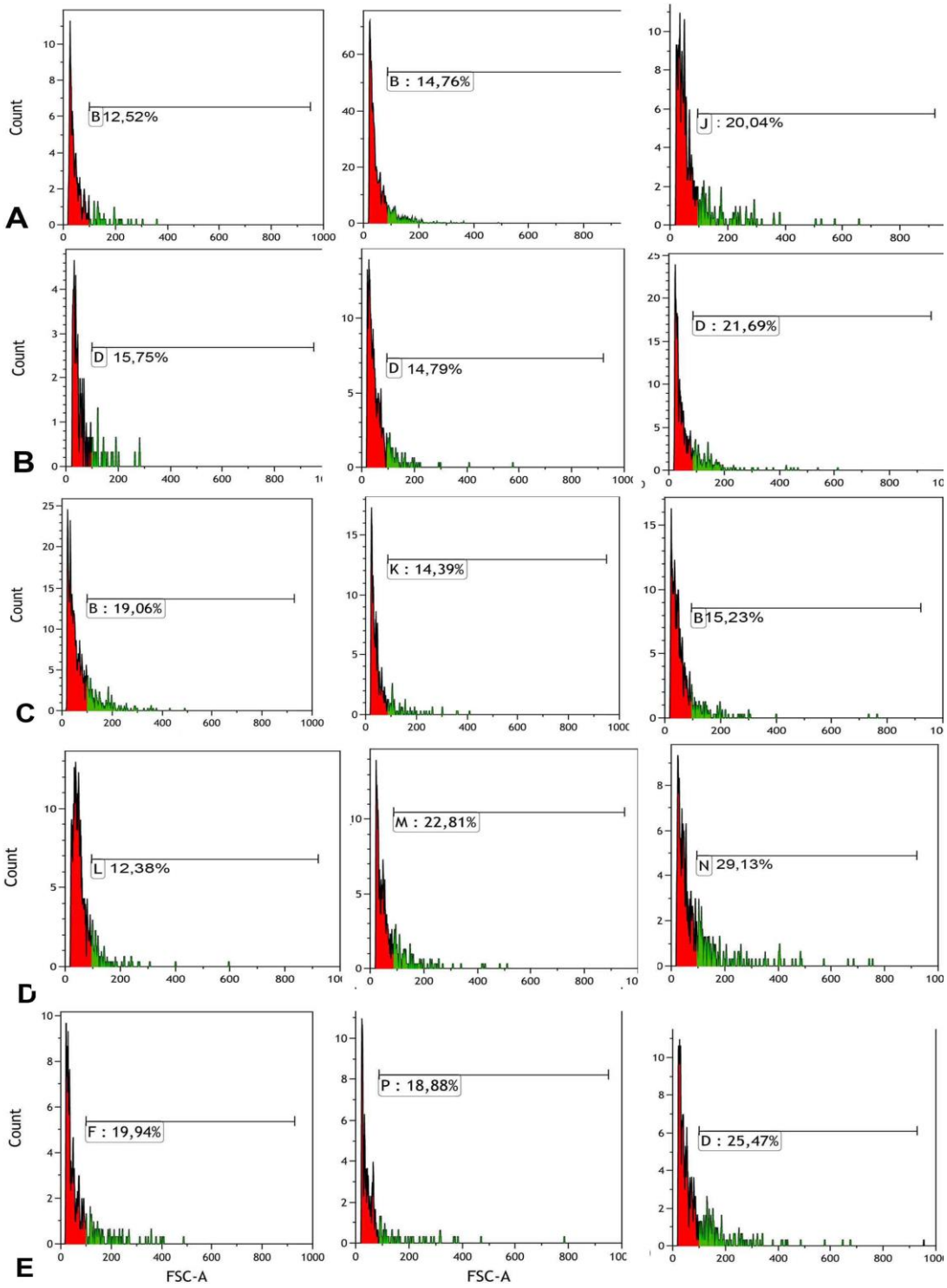

Fig. 6. Histograms of the distribution of cells on the scale of small-angle scattering (FSS, size). A - cells without differentiation inducer after contact with plastic; B - MSC with inducer of differentiation after contact with collagen coating on plastic; C - MSC after contact with the magnesium alloy; D - MSC after contact with calcium-phosphate PEO coating; E - MSC after contact with PEO coating with UPTFE. Flow cytometry, excitation of fluorescence by using an argon laser (495 nm).

In implantation surgery, various types of modern materials, both bioinertic and bioactive, are used. Modification of the surface properties of such materials affects the process of bone tissue regeneration, including protein adsorption and cell proliferation. From these positions, the most promising are metals and alloys with coatings based on calcium phosphates, which are similar in chemical and phase composition to the mineral components of bone tissue. Such methods as sol-gel technology, anodizing, electrodeposition, plasma electrolytic oxidation, plasma spraying, etc. are widely used to treat the surface of implants. These coatings provide biocompatibility and the ability to integrate an implant with bone tissue (Zhou & Gong, 2012, Bakhsheshi-Rad et al., 2016).

The method of plasma electrolytic oxidation used by us makes it possible to obtain calcium-phosphate coatings on magnesium alloys. The solution of this problem involves the creation of a protective layer on low-alloy magnesium alloys that has anticorrosive properties and bioactivity. Magnesium oxide can be the basis on which a hydroxyapatite layer can be applied, that is, the formation of a two-layer coating. We in the bipolar regime of PEO obtained protective coatings on the magnesium alloy MA8, on which occurs the formation of calcium-phosphate compounds, which are close in stoichiometric composition to hydroxyapatite. The PEO layer serves as a matrix where further hydroisapatite crystallization occurs in the electrolyte fluid. This bioactive coating contains hydroxyapatite with a Ca/P ratio of 1.6 close to that of bone (1.67). In this work, for this purpose, calcium glycerophosphate ($C_3H_7O_6P$) Ca $2H_2O$ and sodium fluoride NaF were added to the electrolyte. Calcium glycerophosphate is a calcium salt of phosphoric-glycolic acid, the ion of which contains in its composition both the organic part - the radicals of the triatomic glycerin, and the inorganic part - the orthophosphoric acid anions H3PO4. Organic radicals in the PEO process contribute to a longer burning of the high-temperature plasma discharge, which leads to intense thermolysis of the electrolyte and synthesis in the hydroxyapatite magnesium coating (Song et al., 2008, Razavi et al., 2015). These coatings have a developed porous surface and significantly reduce the corrosion rate of the magnesium alloy.

In the area of bone tissue damage, migration of MSCs and their activation is observed. The decisive factor in the directional differentiation of these cells is the presence of various inducers of osteogenesis, including dexamethasone, ascorbic acid, organic phosphates, in particular β-glycerophosphate, dihydroxyvitamin D3, and some proteins of the family of bone morphogenetic proteins (Madrigal et al., 2014). Such chemical components in the composition of bioactive implant coatings can influence the functional state of MSCs. Analysis of the effect of the test coatings on cells showed, that under conditions of contact roughness and chemical composition of calcium-phosphate coatings are the most important properties determining osteogenic differentiation. With a long period of co-incubation with magnesium without coating, a large area of the cell surface (an active spacing index) and a slight stimulation of the cellular APF testified to the absence of committed stem cells. In other words, MSCs remained largely immature. At the same time stem cells had fibroblast-like morphology and were located mainly on spherulites or their lateral surfaces of samples. While on the calcium-phosphate coating the fibroblast-like morphology of MSCs was practically not observed. So at a size of 20-30 microns the cells were elongated, cylindrical with a clear basophilic cytoplasm and numerous thin processes. Cell cores are round or oval, located more often eccentrically with uniformly reticular chromatin. These morphological features indicated a directed osteogenic differentiation of MSCs.

The International Society for Cellular Therapy defined the minimum criteria determining the properties of MSCs, namely, adhesiveness, multipotency, expression of CD90 receptors (THY1-antigen of thymocytes) and CD106 (endoglin). In this case, the expression of such markers as CD 31, CD 34, CD 45 should be observed in no more than 1-2% of the cells (Salem &Thiemermann, 2010). Freshly isolated MSC of the bone marrow may contain cells carrying the CD34 marker, which is typical of hematopoietic stem cells, but in the presence of differentiation inducers their number decreases. High rates of expression of CD106 were obtained upon contact with calcium phosphate PEO-coating while the other markers decreased. This showed the osteoinductive effect of coatings on MSCs.

This way, protective coatings containing hydroxyapatite with a ratio Ca/P = 1.6 close to the bone tissue index (1.67) were obtained on the magnesium alloy MA8 in the electrolyte solution containing calcium glycerophosphate and sodium fluoride in the bipolar regime of PEO. These coatings have a developed porous surface and significantly reduce the rate of corrosion of the magnesium alloy, which makes it possible to consider them as perspective for bioresorbable medical implants. Such bioactive coatings that modify the surface of magnesium provide a porous surface that promotes the strong attachment of MSCs. Under conditions of contact with cells, the roughness and chemical composition are the most important parameters of calcium-phosphate coatings that determine their effect on the osteogenic differentiation of the cell-tissue level.

ACKNOWLEDGEMENTS

The research is funded by the Russian Science Foundation (project no. 14-33-00009, 2014-2016) and the Government of Russian Federation (Federal Agency for Scientific Organizations).

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