

2010

P1

Adipose tissue was obtained in accordance with the EU ethical standards from patients undergoing liposuction procedures. hADSC cells were obtained using a modified method of *Zuk et al., 2001*, by optimizing the concentration and collagenase type and also the enzymatic digestion time. Briefly, lipoaspirates were washed with phosphate-buffered saline (PBS) to remove contaminating debris and treated with 0.01 % collagenase type I in PBS, supplemented with antibiotic for 60 min at 37°C with gentle agitation. The enzyme was inactivated with culture medium supplemented with fetal bovine serum (FBS). After centrifugation, the cell pellet was treated, to remove red blood cells, with lysis buffer. Cells were seeded in preadipocyte standard medium at a final density of 1.3×10^4 cells/cm² and incubated at 37°C in a humidified atmosphere of 5% CO₂. The culture was monitored by evaluating cell morphology in phase contrast microscopy and by detection of cellular senescence through consecutive passages, emphasizing the β -galactosidase activity in senescent cells. The adipogenic differentiation was shown by Oil Red O staining after exposing the cultured cells to 4 adipogenic media.

In addition we assessed the biocompatibility tests for the scaffolds provided by P7.

P2

P3

Two of the top challenges in the field of hard tissue (HT) engineering remain the development of bio-inspired materials and the increased bioactivity of the biomaterials.

In the first part, P3 developed *new 3D porous scaffolds based on type I fibrillar collagen, human bone morphogenetic protein 4 (BMP-4) and hyaluronic acid (HA)* using freeze-drying method. The aim of this research was to study if the addition of HA and of BMP-4 to collagen scaffolds improves its physical-chemical and morphological properties. The combination of osteoinductive growth factors, such as BMP-4 with scaffolds could provide an appropriate osteoinductive environment for bone cells. The combination of BMP-4 with ECM components involves two different functional concepts: the external pre-integration of osteoinductive BMP-4 and the possible interaction of ECM components with endogenous growth factors. Evaluation of the physical-chemical and morphological characteristics demonstrate that the collagen-HA-BMP-4 scaffolds are suitable for bone tissue engineering purposes.

The second part of the P3 work is devoted to the engineering of smart materials with cluster-like architecture and functionality, very efficient for specific delivery of different bioactive species. P3 reports the development of *materials consisting in undermicronic size*

polymer particles surface-nanostructured with polyamidoamine (PAMAM) dendrimers. The study begins with the PAMAM binding onto the polymer beads, followed by the development of two types of amino-functional clusters. The amino- reactive shell of the nanostructured polymer particles is used to explore their potential to be specifically biofunctionalized. Due to the high reactivity of amino-groups the functionality of the target molecules to be conjugated onto these smart delivery vehicles may be impressively wide, ranging from carboxyl, hydroxyl, epoxy, carbonyl, amines, and nevertheless thiols. Furthermore, the study was continued with the activation of the reactive amino-shell with a heterobifunctional reagent allowing the high-specific immobilization of thiol-containing species. A model thiol-ended peptide, glutathione, has been used to prove the efficiency of the cluster-type delivery system synthesized here. These materials open a generous route of creating solid surface nano-structured undermicronic particles decorated with chemically linked bioactive (and not only) molecules, to be further included in more complex scaffolds.

For all studied materials, the physical-chemical properties were assessed using infrared spectroscopy (FTIR), UV-VIS spectroscopy, x-ray photoelectron spectroscopy (XPS), differential scanning calorimetry (DSC) and thermogravimetry (TG/DTG); the morphological properties were evaluated by scanning electron microscopy (SEM) and by atomic force microscopy (AFM). The hydrodynamic diameters of the obtained particles have been measured by dynamic light scattering (DLS).

P4

1. After processing the TiAlNb alloy the obtained surfaces were type :

- **nanotub** through electrochemical anodizing in $(\text{NH}_4)_2\text{SO}_4$ 1 M + NH_4F 0.5 wt % and glycerol + 4 % H_2O + NH_4F 0.36 wt % solutions (with dimensions dependent on electrolyte and working conditions)

- **grains** through a) laser ablation with a resulting continuous oxide with grains around 3 μm which presented cracks. The oxygen content of oxide film according SEM_EDAX analysis was 16.63 w %; b) through electrochemical anodizing from monomer solution in the presence and without presence of various additions when polymeric hybrid films such as PPy, PPy/Surfactants were obtained

2. The average roughness (R_a) of new structures is much higher especially in the case of polymers films when the roughness is 10 times higher comparing to the uncoated alloy. Regarding the contact angle this parameter is reduced in the case of nanotubular structure comparing with untreated surface with a contact angle (CA = 85.89°). In the case of of polymer

films with surfactants PpyPEG, PPy/Surfactants the contact angle could be reduced in the range of strong hydrophilic character (5°).

3. Oxide films obtained via electrochemical anodizing have better corrosion resistance compared with untreated TiAlNb. A classification of charge transfer resistance (R_{bL}) is as following : nanotub with diameter $40 \div 55$ nm, obtained in glycerol + 4 % H_2O + NH_4F 0.36 wt % ($R_{bL} = 18.68 \cdot 10^6 \Omega \cdot cm^2$) > nanotub with pores diameter ~ 120 nm, elaborated in $(NH_4)_2SO_4$ 1 M + NH_4F 0.5 wt % solution ($R_{bL} = 12.63 \cdot 10^6 \Omega \cdot cm^2$) > natural passive film on Ti6Al7Nb ($R_{bL} = 2.88 \cdot 10^5 \Omega \cdot cm^2$). All types of TiAlNb processing induce a corrosion potential shift in the less electronegative domains comparing with untreated alloy: In the case of granular structures the value are electropositive, for the oxide elaborated via laser ablation being $E_{cor} = 207$ mV. In the case of structure with grains the electrochemical stability seems to be reduced comparing with untreated alloy, and this fact can be explained due to the conductiv character of polymer film compared to non-conductive natural passive film on TiAlNb. The presence of vaious surfactants in the polymerization solution promotes fabrication of compacte polymeric films with a better electrochemical stabiliyt (higher than the stability of Ppy film), the current density values being reduced and charge transfer resistance being improved as following ; PPy/PEG ($R_{bL} = 6.88 \cdot 10^4 \Omega \cdot cm^2$) > PPy/NaPSS ($R_{bL} = 6.3 \cdot 10^3 \Omega \cdot cm^2$) > PPy ($R_{bL} = 3.22 \cdot 10^3 \Omega \cdot cm^2$). Such good electrochemical stability of the polymeric films electrodeposited on TiAlNb open the perspective for coated TiAlNb electrodes to be used as sensors and biosensors.

4. The processing treatment applied on TIALNb alloy lead to better electrochemical stability with various composition morphology and topography able to improve biocompatibility. Experimental results with human osteoblast cell G292 grown on TiAlNb before and after processing proved this fact.

P5

1.5.a. Obtaining of the activated passive films on the supports of Ti and TiAlV by electrochemical methods

Activated passive films (coatings, both on Ti and Ti-6Al-4V ELI alloy support) were obtained by electrochemical method of the cathodic potentiodynamic polarization and chemical method of immersion in physiological solutions with the establishment of optimal activation parameters. Hydroxyapatite was formed and was identified by FT-IR, XRD and Raman methods; SEM micrographs show a suitable structure for the cell adherence on hydroxyapatite coating.

1.5.b. Establishment by the electrochemical methods of short term behaviour of the obtained films on supports of Ti and TiAlV

Behaviour of the coatings obtained from cyclic voltammetry curves. Cyclic polarization curves (obtained in Ringer solution of pH 7.1 and 8.91 and Hank solution of pH 7.4) of the coated biomaterials show a nobler behaviour than of the un-covered biomaterials.

Behaviour of the obtained coatings from Tafel linear polarization. The covered biomaterials had lower corrosion rates than the un-covered biomaterials proving a better protective character.

Behaviour of the obtained coatings from electrochemical impedance spectroscopy. EIS spectra revealed capacitive behaviour, protective, very resistant layer, with increasing thickness in time.

Behaviour of the obtained coatings from the monitoring of the open circuit potentials and corresponding potential gradients. The covered biomaterials presented more electropositive potentials than of the un-covered biomaterials as result of the protective effect of the coating. Open circuit potential gradients due to the non-uniformity of the pH and composition of the physiological solutions have very low values that can not generate galvanic or local corrosion.

1.5.e. Short term morphological characterization of the activated surfaces on the supports of Ti and TiAlV by SEM

The morphology of the coatings deposited on the titanium and Ti-6Al-4V ELI alloy changed in time: the coatings became denser and in the same time presented pores, a favourable structure for the cell adhesion and proliferation.

P6

In this stage P6 synthesised inorganic components for composite scaffolds consisting in vitreous calcium-phosphate and calcium-silicate systems prepared by quickly undercooling of melts to room temperature ($2\text{P}_2\text{O}_5 \cdot \text{CaO} \cdot 0.05\text{Li}_2\text{O}$, $x\text{Ag}_2\text{O} \cdot (1-x)[2\text{P}_2\text{O}_5 \cdot \text{CaO} \cdot 0.05\text{Li}_2\text{O}]$, $x\text{TiO}_2 \cdot (1-x)[\text{P}_2\text{O}_5 \cdot \text{CaO}]$ and $x\text{Ag}_2\text{O} \cdot (1-x)[0.01\text{TiO}_2 \cdot 0.99(\text{P}_2\text{O}_5 \cdot \text{CaO})]$), and via the sol-gel route gel ($0.55\text{SiO}_2 \cdot 0.41\text{CaO} \cdot 0.04\text{P}_2\text{O}_5$, $0.45\text{SiO}_2 \cdot 0.245\text{CaO} \cdot 0.06\text{P}_2\text{O}_5 \cdot 0.245\text{Na}_2\text{O}$ and $0.445\text{SiO}_2 \cdot 0.445\text{P}_2\text{O}_5 \cdot 11\text{ZnO}$ – molar composition). In order to obtain vitroc ceramic samples, the as-prepared samples were heat treated at temperatures determined according to the results delivered by differential thermal analysis. X-ray diffraction data indicate that the as prepared samples are in vitreous state and after the applied heat treatment they are partially crystallised. The surface topography and morphology of $0.445\text{SiO}_2 \cdot 0.445\text{P}_2\text{O}_5 \cdot 11\text{ZnO}$ samples were investigated by atomic force microscopy. The grain roughness heights around $3\mu\text{m}$, and areas up to $8.63\mu\text{m}^2$ are convenient for the attachment of biomolecules.

P7

P7 has synthesized an alginate hydrogel-based scaffold. Alginate gelation occurs almost instantaneously in the presence of divalent cations Ca^{2+} , Mg^{2+} , Sr^{2+} , Ba^{2+} which interact with the carboxyl groups of glucuronic acid forming ionic bridges. For this reason, the consistency and uniformity of the structure of the resulting hydrogel may be controlled. The decrease in the desired reaction rate can be achieved by controlling the concentration of calcium ions. The basic idea was to replace all the dissociated CaCl_2 salt in aqueous medium with calcium gluconate (calcium gluconate). Basically we started with glucono- δ -lactone and calcium carbonate (insoluble in water). The new formed gluconic acid reacts with calcium carbonate to form calcium gluconate. Na alginate concentration and CaCO_3 allow the control of the rate of crosslinking. The purity of the product was evaluated as well as the toxicity of the degradation products. At the same composition, there was identified a significant difference between the consistency of hydrogels obtained in aqueous medium vs those prepared in the culture medium with or without serum.