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Albumin coatings by alternating current electrophoretic deposition for improving corrosion resistance and bioactivity of titanium implants

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ABSTRACT

Although Ti alloys are generally regarded to be highly corrosion resistant, inflammatory conditions following surgery can instigate breakdown of the TiO_2 passivation layer leading to an increased metal ion release. Furthermore proteins present in the surrounding tissue will readily adsorb on a titanium surface after implantation. In this paper alternating current electrophoretic deposition (AC-EPD) of bovine serum albumin (BSA) on Ti6Al4V was investigated in order to increase the corrosion resistance and control the protein adsorption capability of the implant surface. The Ti6Al4V surface was characterized with SEM, XPS and ToF-SIMS after long-term immersion tests under physiological conditions and simulated inflammatory conditions either in Dulbecco's Modified Eagle Medium (DMEM) or DMEM supplemented with fetal calf serum (FCS). The analysis showed an increased adsorption of amino acids and proteins from the different immersion solutions. The BSA coating was shown to prevent selective dissolution of the vanadium (V) rich β-phase, thus effectively limiting metal ion release to the environment. Electrochemical impedance spectroscopy measurements confirmed an increase of the corrosion resistance for BSA coated surfaces as a function of immersion time due to the time-dependent adsorption of the different amino acids (from DMEM) and proteins (from FCS) as observed by ToF-SIMS analysis.

1. Introduction

Regarding the interactions of metal alloy implant surfaces with the biological environment, it is well known that a layer of proteins is adsorbed on the surface immediately after contact with human blood. These surface-specific adsorbed protein layers act as a contact for cell attachment, thereby governing the cellular response and eventually the bioactivity of the implant surface [1]. This protein adsorption has been reported to enhance the corrosion resistance of implant materials [2–5]. The latter is of particular importance because immediately following the implantation of medical devices such as metal prostheses, an inflammatory response is triggered attracting phagocytes such as neutrophils (acute phase) and macrophages (chronic phase) to the implantation site which secrete highly reactive oxygen species at the implant/tissue interface and can decrease the local pH down to 5 [6–9]. It has been previously shown that when the titanium (Ti) alloy is exposed to such aggressive physiological environment, the oxide stability will be affected, resulting in increased metal ion release and etching of the vanadium (V) rich β-phase of the alloy [10,11].

Albumin is the protein with the highest concentration in blood serum (60%) and it is known to promote adsorption of biomolecules [12–14] as well as hydroxyapatite nucleation [15], two essential processes for a successful osseointegration of an implant. The presence of bovine serum albumin (BSA) has also been shown to increase the corrosion resistance of Ti6Al4V, and this in different electrolytes [16–18]. Given this enhanced bioactivity and corrosion resistance, it is worthwhile to investigate techniques forming thick albumin layers on Ti6Al4V. As such, electrophoretic deposition (EPD) is of high interest. This colloidal technique is already widely investigated in material science, for the processing of inorganic bioactive coatings, as well as in biochemistry for the manipulation of proteins, enzymes, cells and colloids [19–24]. Nowadays, EPD is gaining more and more attention as a biomolecule coating technique, because of its ability to obtain high purity deposits with a variety of thicknesses on complex-shaped metal substrates in a cost-effective way. In essence, EPD is a two-step process including the migration of the charged particles or molecules by applying an external electric field (electrophoresis), followed by the accumulation and deposition on the electrode surface [25]. EPD of inorganic materials is most often carried out applying direct current (DC) fields. In order to avoid hydrolysis and Joule heating at higher voltages, either non-aqueous solvents or low voltages are applied. The need for aqueous suspensions when working with biomolecules, however, has triggered the use of alternating current (AC) fields [23,24]. It has been shown

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that for sufficiently high frequencies the electrolysis of water (and concomitant gas bubble formation and pH shift at the electrodes) can be avoided, so that in combination with unbalanced asymmetrical AC signals, a net drift of charged particles can be realized because the particle velocity increases more than linearly with the field strength [26,27].

Therefore, in this study, AC-EPD was used to deposit BSA on the Ti6Al4V surface. The effect of this BSA layer on the corrosion behavior of Ti6Al4V under simulated inflammatory conditions and the presence of H_2O_2, as well as on the adsorption of proteins or amino acids was studied in long-term immersion tests up to 28 days.

2. Material and methods

2.1. AC-EPD of the albumin layer

The BSA used in this study was obtained from Sigma Aldrich (lyophilized powder, ≥96% (agarose gel electrophoresis)). Fresh suspensions of 4 mg/mL BSA in milliQ water were prepared for each experiment. A Ti6Al4V rod (Ø 22 mm) was cut into disks, which were ground with a P1200 SiC paper (Microcut, Buehler) and cleaned ultrasonically in ethanol and acetone for 15 min each. AC-EPD was carried out at ambient temperature in an Ø 20 mm horizontal deposition cell, the Ti6Al4V electrode was placed at the bottom while polished stainless steel served as a counter electrode at a distance of 10 mm. The voltage-controlled AC signal was generated using a high resolution digital-to-analog signal output module (NI9269, National Instruments) coupled to a bipolar amplifier (PZD 700M/S, Trek Inc.). An asymmetric triangular AC-signal with a 50 Hz frequency and an asymmetry of 3, was applied [27]. The peak-to-peak amplitude was set to 100 V and a 2 V DC-offset was used. Afterwards, the samples were rinsed in ethanol and dried with nitrogen gas.

2.2. Immersion in DMEM and FCS

The effect of the experimental BSA layers on the corrosion resistance, adsorption of amino acids and formation of calcium phosphates (Ca-P) on Ti6Al4V was examined during long-term immersion tests in 50 mL Dulbecco's Modified Eagle Medium (DMEM, Merck KGaA), a cell culture medium with similar chemical composition to serum or SBF (simulated body fluid) supplemented with amino acids [28]. Immersion was performed either under physiological conditions at pH 7 for a total duration of 28 days (control), or under simulated inflammatory conditions at pH 5 (through the addition of H_2O_2 (37%, Sigma Aldrich)) for 14 days [11], followed by immersion under normal physiological conditions at pH 7 for the remaining 14 days, to simulate the expected duration of inflammatory reactions in clinical cases [11,29]. To investigate the effect of protein adsorption on the experimental BSA coatings, the samples were immersed in DMEM supplemented with 20% fetal calf serum (FCS; Merck KGaA). Once again, a comparison between immersion under physiological conditions and simulated inflammatory conditions was made.

Samples were incubated at 37.5 °C in a humidified atmosphere of 95% air and 5% CO_2 and removed for analysis every 7 days, and this up to 3 months. For humidification, a fixed cup was filled with high-purity water. During immersion, the solution was refreshed every 7 days, and in order to prevent evaporation of the solution, the samples were covered with aluminum (Al) foil. After immersion, the samples were washed with distilled water and dried with nitrogen gas. The immersion solutions of the BSA coated samples were analyzed with ICP-OES to determine the metal ion release. However, all metal ion concentrations (DMEM and DMEM + FCS) were under the detection limit of the ICP device, therefore the ion release will not be shown in this manuscript. The detection limit depends on the element(s) being analyzed and the sample matrix. In case of DMEM immersion solution the detection limit can be calculated as follows: Ti ~0.01 mg/L; Al ~0.018 mg/L; V ~0.016 mg/L. In addition, the limit of determination is calculated as three times the limit of detection. The metal ion release from the uncoated Ti6Al4V alloy has been reported elsewhere [11]. So immersion under inflammatory conditions of the uncoated Ti6Al4V leads to increased Ti (1.2 μg/L), Al (0.1 μg/L) and V (0.15 μg/L) concentrations even after 7 days.

2.3. Electrochemical impedance spectroscopy (EIS) measurements

Electrochemical impedance measurements were carried out using a potentiostat (AUTOLAB model PGSTAT 302N) with a conventional three-electrode system, whereas the Pt foil served as counter electrode and an Ag/AgCl (3 M KCl) electrode as reference electrode. During the measurements, the samples were immersed in 100 mL of DMEM heated up to 37.5 °C with a thermostat (Thermostat HAAKE F3). A sine wave with an amplitude of 10 mV was applied to the Ti6Al4V working electrode, polarized at the previously measured open circuit potential. The open circuit potential was measured before versus the Ag/AgCl electrode (3 mol dm^{-3} KCl) for 30 min. The spectra were acquired in the frequency range of 100 kHz to 10 MHz.

2.4. Characterization of the BSA surface

To investigate the BSA coating morphology prior to immersion in DMEM, SEM (Nova NanoSEM 450, FEI) was performed using a standard high-vacuum setting, but applying beam deceleration mode in order to avoid damage of the organic coating. Hence, the beam energy (6–7 keV) was reduced by applying a 3–4 keV stage bias field resulting in an effective landing energy of 3 keV.

After immersion in DMEM (with or without FCS), the samples were investigated by SEM (Hitachi S4800 FESEM) to see the effect of the BSA coating on Ca-P formation. Furthermore, XPS measurements were used to determine the surface chemistry (PHI 5600; Al Kα radiation (1486.6 eV; 300 W) for excitation). For this, three different samples with five spots each were measured. In order to characterize the adsorbed amino acids and proteins, positive and negative static SIMS measurements (ToF-SIMS 5 spectrometer, ION-ToF) were performed on three different spots on each of the immersed samples. The spectra were recorded in high mass resolution mode ($m/\Delta m > 8000$ at Si and >12,000 at m/z 200) with a pulsed 25 keV Bi_3^+ liquid-metal ion beam. Prior to analysis, the spectra were calibrated to the positive CH_3^+, $C_2H_3^+$, $C_3H_5^+$, $C_4H_9^+$ and $C_7H_7^+$ peaks, respectively.

3. Results

3.1. Interaction with DMEM

To investigate the BSA coating morphology before the samples were immersed in DMEM, a Nova NanoSEM 450 from FEI was used with standard high-vacuum setting but in beam deceleration mode in order to avoid damage of the organic coating (Fig. 1).

After immersion in DMEM under physiological conditions, control Ti6Al4V alloy samples generally have a smooth surface which reveals the microstructure with α and β phases (results not shown). After immersion in DMEM under simulated inflammatory conditions for 14 days, however, a selective etching of the phase boundaries can be observed (Fig. 2A and B). The TiO_2-H_2O_2 interaction has led to accelerated corrosion of the V-rich β-phase and an increased metal ion release of Ti, Al and V, as confirmed by ICP-OES in our previous report [11]. In contrast, whenever a BSA layer is deposited on the Ti6Al4V surface by means of AC-EPD, only the additional protein layer can be observed. No dissolution or etching of the phase boundaries is seen, neither after immersion under physiological conditions (Fig. 2C and D) nor after immersion under simulated inflammatory conditions for 14 days (Fig. 2E and F). The decreased pH and additional H_2O_2 under simulated inflammatory conditions, however, leads to a surface roughness in the nanometer scale. In accordance with these SEM observations, no detectable