In vitro electrochemical behaviour of Chitosan-PEG coatings obtained on Ti6Al4V by dip coating

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Abstract. In the present study, coatings of Chitosan - Polyethylene Glycol was obtained by dip coating on Ti6Al4V from polymer mixtures (30-70, 50-50, 70-30). The aim of our study was analysed their ability to formation of apatite and their electrochemical behaviour in the presence of a simulated physiological fluid. The coating properties were evaluated by electrochemical impedance, atomic absorption, SEM and FTIR techniques. The results showed that surfaces coated with a 50-50 ratio of chitosan PEG stimulate the formation of calcium phosphates on the surface of the material. On the other hand, it was found that the number of layers generate a very significant effect on the resistance to the degradation of the alloy in the presence of SBF, generating an increase of up to 1 and 3 orders of magnitude in the impedance of the coating of 5 and 7 layers respectively compared to the Ti6Al4V alloy.

1. Introduction
In recent years, titanium and its alloys have developed into the key materials for orthopedic and dental implants surgery, owing to their excellent bulk of properties [1-3]. Nevertheless, these materials are associated with certain clinical challenges. For this reason, a wide number of works have recently been developed to improve the properties of the titanium and its alloys. Some of these studies have focused on improving the oseo-conductive capacity. While others studies have been focused on reducing the bacterial adhesion on the surface of the implant [4-7].

One of the ways to achieve this has been the modification of the layer of titanium oxide that is formed naturally on the surface of these materials. This modification has allowed improving the roughness of the surface thus favouring the cellular response and also the ability to integrate the material to the bone. [8-10]. Another alternative is the chemical treatment with solutions of NaOH or KOH, this type of modification has allowed to incorporate ions (OH⁻) to the surfaces of titanium, which favour the nucleation of calcium phosphates, as is the case of hydroxyapatite [11]. Other authors have chosen to modify the composition of the titanium surface by the growth of calcium phosphates or other compounds that resemble the composition of the bone mineral matrix, in order to generate a surface whose composition favours osseointegration of the implant [12-14]. Likewise, there are works where the main materials of the superficial modification are the polymeric. These materials, when implanted and interact with the physiological environment, initiate a slow degradation process which allows the release of substances that are metabolized by the cells and thus allow to favour the conditions to improve cell adhesion and proliferation [15]. On the other hand, some polymeric materials manage to reduce the adhesion of bacteria, as is the case of chitosan and PEG [16,17].
The aim of this study was to evaluate the effect of the concentration and the number of layers of chitosan PEG coatings obtained by dip coating on Ti6Al4V in the capacity of apatite’s formation and the electrochemical behaviour in simulated body fluid.

2. Experimental

Ti6Al4V cylindrical disc with 14mm diameter and 3 mm thick were ground with silicon carbide paper in successive grades from 120 to 1200 grit. Then were polished with a slurry of alumina (particle size 0.5μm) with cotton cloths. Subsequently the disc was cleaned by ultrasonically in ethanol for 15 minutes.

An alkaline treatment was performed in order to activate the surface, which was immersed in 10M NaOH solution for 24 hours at 60°C in a hydrothermal bath. The samples were washed in distilled water and dried at room temperature. In order to stabilize the solid formed, a heat treatment was carried out with a heating ramp of 25°C to 450°C in 43 minutes, maintaining this temperature for 1 hour.

The polymer solutions of Chitosan - polyethylene glycol was prepared from individual solutions of chitosan and PEG, dissolved in 0.5M acetic acid and water respectively. Subsequently they were mixed to form a single homogeneous solution. Three solutions were obtained with 30/70, 50/50 and 70/30 Chitosan-PEG ratios. The amounts of chitosan and PEG used for the preparation of the polymer ratios are shown in Table 1.

| Table 1. Reagents to prepare different ratios of chitosan polyethylene glycol. |
|-----------------|------|------|-----------------|-----------------|
| Chitosan (g)    | Acetic acid (ml) | PEG (g) | Distilled water (ml) | Chitosan-PEG Ratio |
| 0.09            | 9    | 0.21 | 21              | 30:70           |
| 0.15            | 15   | 0.15 | 15              | 50:50           |
| 0.21            | 21   | 0.09 | 9               | 70:30           |

The polymer solutions Chitosan-PEG were used to coat the discs of the alkaline treated Ti6Al4V alloy by immersion and extraction of the substrate using the Dip Coating technique. The immersion time of the samples in the polymer solution was 10 seconds, and then a drying was carried out in a preheated oven at 50°C for 30 minutes. The number of layers made on the specimens was 3, 5 and 7 layers. The coated samples were immersed in polypropylene containers with 30ml of a Hank's solution (NaCl 8.0, KCl 0.4, MgSO4·7H2O 0.2, CaCl2 0.185, Na2HPO4 0.046, KH2PO4 0.06, Glucose 1.0, NaHCO3 0.35g/L) for 180 hours to induce apatite formation in a hydrothermal bath at 37°C.

The coating morphology was analysed by scanning electron microscopy before and after immersion on the Hanks solution. The functional groups present in the coating were analysed by Fourier transform infrared spectroscopy. The chemical reactivity of the coatings was evaluated by quantifying the amount of calcium adsorbed by the surfaces of Chitosan PEG by analysing the variation of the calcium concentration in the Hanks solution, using the atomic absorption technique with which it was possible determine the calcium present the Hanks solution after the immersion test. Finally, the electrochemical behaviour of the samples was evaluated simulating physiological conditions in hanks solution at 37°C. The tests were electrochemical impedance spectroscopy, for which a sweep of frequencies of 0.01 to 100 000Hz was applied applying 30mV rms. A potentiostat-galvanostat Gamry 600 was used for the realization of this tests, using a flat cell composed of three electrodes in which the Ti6Al4V discs were used as working electrode, graphite as counter electrode and Ag/AgCl 3M KCl as reference electrode. The exposure area of the sample was 0.63cm². The samples were previously immersed in the solution for 5 minutes to allow stabilization of the system.

3. Results and discussion

Figure 1 shows the morphology of the coatings of chitosan polyethylene glycol. As can be seen the number of layers the coating becomes more homogeneous and decreases the number of cracks present on the surface. White spots observed as precipitates on surfaces are associated with chitosan particles that were not completely dissolved.
Figure 1. Morphology of surfaces Chitosan PEG. 5 coatings a) 30/70 b) 50/50 c) 70/30.

Figure 2 shows the analysis of the functional groups present on the developed chitosan polyethylene glycol surfaces. There is an asymmetric and flexion between 2860 and 3350 cm\(^{-1}\) band, due to the hydroxyl and carboxyl groups present in chitosan rings, also NH deformation associated with chitosan around 1617 and 1637 cm\(^{-1}\). The presence of these bonds favours primary amide between the calcium cations, the phosphates and the surface of the biomaterial, which could cause an increase in the nucleation and the precipitation of the calcium phosphates in the surface.

Figure 2. Infrared spectrum the ratio 30/70, 50/50, 70/30 Chitosan: PEG, 5 layers.

Figure 3. Concentration of calcium present in the fluid for different polymer ratios.

As can be seen in Figure 3, exist is a variation in the calcium concentration of aliquots with the Hank's solution, due to the phenomenon of adsorption on this metal ion by test samples, which reduces its concentration in each aliquot. It should be noted that the coatings that adsorbed the most calcium were 50/50 blend, this mean that it is the best combination of properties the polymer blend; By the chitosan having a porous matrix makes it more apt to interact with the fluid because it increases its surface area, favouring the nucleation and growth of phosphates [18]. In turn the PEG is a water-soluble polymer, which readily allows the activation of oxygen species which could provide an electron-bonding network to adsorb Ca\(^{2+}\) by electrostatic interaction to form apatite nucleation sites [19].

Figure 4. Compositional analysis of chitosan polyethylene glycol coatings. (a) 30/70, (b) 50/50, and (c) 70/30.

The composition and morphology of the chitosan polyethylene glycol coatings are shown in Figure 4. It can be seen that after 7 days of immersion in Hanks the coatings show precipitates whose composition indicates a possible formation of calcium phosphates on the surface. In addition, it can be observed that other elements appear in the composition which are associated with the precipitation of
other salts that are present in the Hanks solution. It is noteworthy that the coatings with the 50/50 and 70/30 ratios are those with the highest number of calcium phosphate precipitates, because chitosan by its cationic nature allows it to interact with negative ions as well as phosphate groups (PO43-) present in Hank's solution.

In Figure 5(a), at high frequencies (10^4-10^5Hz), the solution resistance presents a pseudocapacitive behaviour corresponding. In very low frequencies (0.01-1Hz) the 50/50 and 70/30 ratios have the highest electrochemical impedance associated with a highly resistive behaviour, this behaviour corresponds to the interface coating-material base Ti6Al4V. For the 30/70 ratio a very stable behaviour is observed in low and medium frequencies, which indicates the presence of a passive, compact and very stable layer [20].

In Figure 5(a) behaviour solution is observed at high frequencies (10^4-10^5Hz), also a resistive behaviour for 3 layers and capacitive one for 5 and 7 layers. This behaviour happens in the interface coating-physiological solution where the resistivity for 3 layers refers to a compact layer, and the capacitive effect for 5 and 7 layers, it is due to a greater interaction of the polymer layer with Hank's solution due to a larger area of exposure by the porous chitosan structure for a 50/50 ratio. In addition, it is observed that at low frequencies (0.01-1Hz) it is mainly observed the behaviour of the base material with the coating where the resistance of coating increases as the number of layers increases, where the highest is for 7 layers and the lowest for 3 layers.

Figure 5. Bode diagram. (a) different Chitosan / PEG ratios, (b) different number layers.

Figure 6. Nyquist diagram (a) different Chitosan/PEG ratios, (b) different number layers.

Figure 5 shows the bode plots for the chitosan-PEG coatings. It can be observed that in the high frequency range (10^4-10^5Hz) the resistance to the solution presents different values depending on the type of coating being evaluated. This change in resistance behaviour is associated with the solubility of PEG in the electrolyte. On the other hand, in the low frequency range (10^-2-10^0Hz) it is observed that
the variation in the different PEG chitosan ratios can generate an increase of up to one order of magnitude in the impedance of the Ti6Al4V alloy (Figure 5 (a)), while increasing the number of layers of the coating, the impedance increases up to 4 orders of magnitude (Figure 5(b)).

As can be seen in Figure 6(a), the diameters of the semicircles in the Nyquists diagram increase with increasing concentration of chitosan, this behaviour can be associated with the low solubility of chitosan. The effect of increasing the number of coating layers on the impedance is shown in Figure 6(b), where we can see that the 7-layer coating has a larger dome diameter, which is associated with a high resistance to degradation. In addition, the increase in the height of the domes indicates that the coating of chitosan polyethylene glycol tends to be highly capacitive, which could favour the processes of electrical interaction between the surface and the anionic and cationic species of the Hanks solution.

4. Conclusions
The coatings of PEG chitosan showed morphology in function of the number of layers, where the highest homogeneity was achieved after depositing 7 layers on the surface of the Ti6AL4V alloy. Under the conditions of study, it was achieved after depositing 7 layers surface of the Ti5Al4V alloy. Under the conditions of study, it was possible to determine that the coatings with the 50/50 ratio favour the formation of the adsorption o calcium phosphates on the surface, besides these two coatings presented highest resistance to the degradation in the presence of the Hanks solution.

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