CORROSION AND CYTOTOXIC BEHAVIOUR OF PASSIVATED AND TIN-COATED 316L STAINLESS STEEL

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Abstract. In this work the corrosion and cytotoxic behaviour of surgical austenitic AISI 316L stainless steel specimens have been studied. The material was tested in different surface conditions: (i) bare, non-passivated; (ii) passivated in HNO_3 (30%v) solution at room temperature for 10 minutes; (iii) passivated as described in (ii) and TiN-coated. All the specimens were immersed for a 28-day period in Hanks' solution at 37 ºC. The corrosion behaviour was evaluated by potentiodynamic polarization and electrochemical impedance spectroscopy (EIS). TiN coating was produced using a physical vapour deposition method. *In vitro* cytotoxicity assays have been conducted to assess the biocompatibility of the specimens. As inferred from EIS results, the bare non-passivated specimens presented a highly capacitive behaviour after the first day of immersion in Hanks' solution. No significant alterations were observed for increasing immersion periods. The passivated steel showed similar behaviour during the first week of immersion with high phase angle values through a wide frequency range. However, after 28 days, less capacitive result was obtained. Passivated TiN-coated specimens presented a slight increase in the localized corrosion resistance as indicated by potentiodynamic polarization curves. *In vitro* cytotoxicity results showed that the AISI 316L was non-cytotoxic either TiN-coated or not.

Keywords: 316L, biomaterials, TiN, cytotoxicity, corrosion

INTRODUCTION

Austenitic stainless steels are commonly used for biomedical applications but present poorer corrosion resistance when compared to other metallic biomaterials such as cobalto-chrome and titanium alloys [1,2]. There are several reports in the literature on the localized corrosion of stainless steel implants [3-5]. Corrosion products released to the human body may lead to infectious reactions that make the patient undergoes additional suffering ultimately leading to the implant failure [6]. However, these materials are still used as implants due to low cost and suitable mechanical resistance [7]. Surface modification techniques and coatings deposition may enhance both corrosion resistance and biocompatibility of stainless steel. Titanium nitride ceramic hard coatings due to the intrinsic biocompatibility and high wear and corrosion resistances are suitable for biomedical uses as protective surface films [8,9]. Previously reported results indicated that non-passivated TiN-coated 316L specimens might undergo faster deterioration process than bare specimens when immersed in Hanks' solution [10]. The passivation of the stainless steel surface in acidic solution may be a cheap and effective method to improve its corrosion resistance [11-13]. So, the combination of passivation and TiN films may produce beneficial effects on the corrosion resistance and biocompatibility of austenitic stainless steel.

The aim of this work was to evaluate the corrosion resistance of 316L stainless steel (ASTM F-138) in three discint conditions: bare non-passivated, passivated in nitric acid solution, and passivated TiN-coated. Electrochemical impedance spectroscopy and potentiodynamic polarization tests were used as investigating techniques. The biocompatibility of the materials was determined using a cytotoxicity assay.

EXPERIMENTAL

Material. The chemical composition of the 316L stainless steel used in this work is given in table 1.

Table 1. Chemical composition of the 316L stainless steel (mass percentage).

C	Si	P	S	Cr	Mn	Cu	Ni	Mo	N	Fe
0,007	0,370	0,007	0,002	17,40	1,780	0,030	13,50	2,120	0,070	Bal.

Passivation treatment

Some 316L specimens were passivated in nitric acid solution (30% v/v) at room temperature during 10 minutes. After passivation the specimens were rinsed in deionized water and dried under a hot air stream.

TiN coating

The TiN coatings produced by PVD method were deposited on passivated 316L specimens, in a vacuum chamber. The deposition rate was 0.8 - 1.0 μm/hour. The resulting film thickness was in the range of 2-3 μm.

Electrochemical measurements

Bare 316L specimens were prepared by epoxy cold resin mounting, leaving an exposure area of 1.7 cm^2 to the electrolyte. The surface was prepared by sequential grinding with silicon carbide paper up to #2000 finishing, followed by mechanical polishing with diamond paste of 1 μm. Passivated and passivated TiN-coated 316L specimens, with an exposure area to the electrolyte of approximately 1.7 cm^2, did not undergo any surface grinding or polishing. All the specimens were degreased with acetone and rinsed in deionized water. After surface preparation and prior to the electrochemical tests, all the specimens remained immersed for 24 hours in Hanks' solution, naturally aerated at 37 ^oC.

A three-electrode cell arrangement was used for the electrochemical measurements, with a saturated calomel reference electrode (SCE) as reference electrode and a platinum wire as the auxiliary electrode. The EIS tests were carried out in triplicate to evaluate results reproducibility. The electrolyte used to simulate the physiological medium was Hanks' solution, naturally aerated (pH=6.8). All specimens were immersed in the electrolyte for a period of 28 days.

EIS measurements were carried out with a frequency response analyser (Solartron SI-1255) coupled to a potentiostat (EG&G PARC 273A). All EIS measurements were performed in potentiostatic mode at the corrosion potential (E_{corr}).

The amplitude of the perturbation signal was 10 mV, and the investigated frequency range varied from 10^5 Hz to 10^{-2} Hz with an acquisition rate of 6 points per decade.

A potentiostat/galvanostat EG&G 273A was used for the polarization measurements. Potentiodynamic polarization curves were obtained after 28 days of immersion in Hanks' solution at room temperature, using a scanning rate of 1 $mV.s^{-1}$. The measurements started at -800 mV_{SCE} and finished at 3000 mV_{SCE}.

Cytotoxicity assay

The method is based on the quantitative assessment of surviving viable cells upon exposure to the toxic agent, by incubation with the supravital dye tetrazolium compound MTS (3-(4,5-dimethyltiazol-2-yl)5-(3-carboxymethoxyphenil)-2-(4-sulphophenil-2H-tetrazolium) and an electron coupling reagent PMS (phenazine methosulphate). MTS is bioreduced by cells into a formazan product that is soluble in tissue culture medium, then colorimetric analysis of the incorporated dye is performed. The amount of MTS, the marker of cell viability, taken up by the population of cells, is directly proportional to the number of viable cells in the culture. The test sample inducing cell toxicity is measured over a range of extract concentrations of the biomaterial and the concentration yielding a 50% reduction in MTS uptake is taken as the toxicity parameter.

The cytotoxicity assay of bare non-passivated 316L and TiN-coated 316L was carried out according to ISO 10993-part 5. The cell line recommended by ISO is a preferred established cell line obtained from recognized repositories as American Type Culture Collection (ATCC). Chinese hamster ovary cells culture (ATCC CHO K1) was used in this investigation. Cytotoxicity index ($IC_{50\%}$) was estimated by curve interpolation as the biomaterial extract concentration resulting in 50% inhibition of MTS uptake after plotting the mean percentage of surviving cells against the concentration of the extract (%). In this work phenol solution 0.3% and titanium plate were used as positive and negative control, respectively.

RESULTS AND DISCUSSION

Bode plots (phase angle) for bare non-passivated 316L, passivated 316L and passivated TiN-coated 316L are shown in Fig. 1 for 1 day and 28 days of immersion in Hanks' solution.

The EIS results of bare 316L SS for one day of immersion show a highly capacitive behaviour at low frequencies (Fig. 1a). The phase diagram presents two time constants that are not clearly separated due to some overlapping, but it is possible though to identify a shoulder around 10 Hz. The second time constant at lower frequencies shows phase angles approaching –80°. Stainless steels have a passive superficial layer consisting mainly of Cr_2O_3 [14] that accounts for their relative high corrosion resistance. It is likely that the two time constants are due to the response of the passive layer (still with few defects due to the short immersion period) and to charge transfer reactions at surface of the 316L substrate. After 28 days of immersion (Fig. 1b), the two time constants are more clearly separated. The peak related to the first time constant (at intermediate frequencies) shifts to higher frequencies and decreases with time from one day to 28 days, indicating the improvement of the oxide film protective characteristics, while the second one, at lower frequencies, remains highly capacitive suggesting that significant deterioration did not occur during the whole immersion period. This is likely due to blockage of the defective areas in the oxide as the film grows with time.

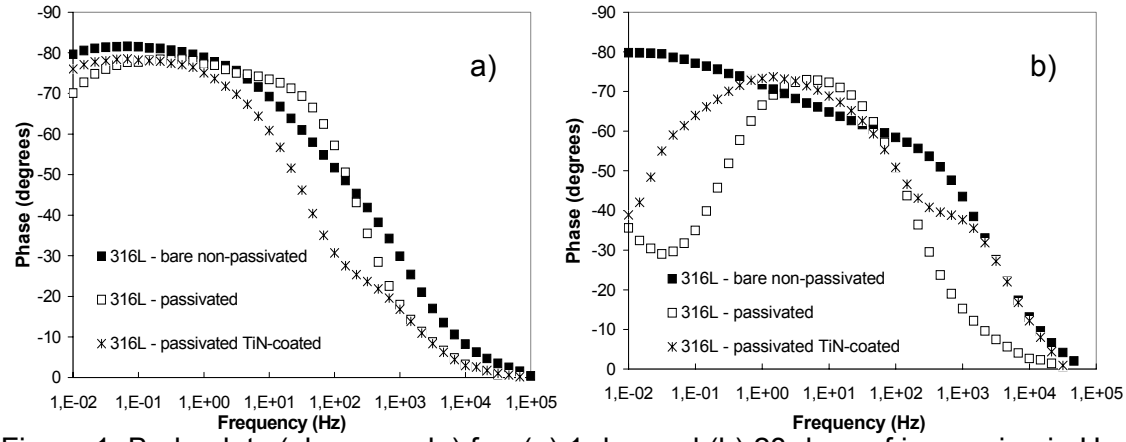

Figure 1. Bode plots (phase angle) for: (a) 1 day and (b) 28 days of immersion in Hanks' solution at 37ºC.

The passivation treatment significantly altered the electrochemical behaviour of the 316L stainless steel, especially for 28 days of immersion. The EIS data corresponding to the passivated 316L specimen for 1 day shows that the time constant peak at medium frequencies is higher and also occurs at higher frequencies comparatively to the bare 316L specimen. However, slightly less capacitive angles are associated to the time constant at lower frequencies for the passivated specimen (Fig. 1a). These results could be explained by the growth of a porous film on the passivated specimen, favouring the charge transfer processes at the porous base. The diagram obtained after 28 days support this hypothesis showing a significant decrease in the phase angles at lower frequencies followed by the indication of a new time constant at frequencies below 0.1 Hz. This can be ascribed to localized attack of the passivated specimen. It is possible that during passivation in the nitric acid solution the fast oxide growth tends to develop a film with more internal defects than that slowly and naturally formed.

After 1 day the diagram for passivated TiN-coated specimen resembles that of the bare non-passivated one especially for the low frequency region (Fig. 1a). There is a capacitive time constant characterized by a shoulder near 10 Hz that extends up to 0,01Hz. This time constant is less capacitive when compared to other specimens (bare non-passivated and passivated ones). It is well known that TiN films produced by PVD methods have a structure with pinholes that are inherent to the coatings process [15]. The penetration of the electrolyte solution through the coating pinholes probably accounts for the attack of the metallic substrate as the immersion time increases. This effect was reported by Yang et al. [15].

Bode plots as $|Z|$ vs ω for the bare non-passivated 316L, passivated 316L and passivated TiN-coated 316L obtained after 1 and 28 days of immersion are shown in Fig. 2. It is evident from the diagrams that the impedance related to the passivated TiN-coated specimen is lower than that of the bare non-passivated and passivated ones. Additionally the impedance value slightly decreased with increasing immersion times as seen in Figures 2 a) and 2b). This behaviour reflects the results on the phase diagrams

also suggesting the attack of passivated metallic substrate on the base of the coatings pinholes.

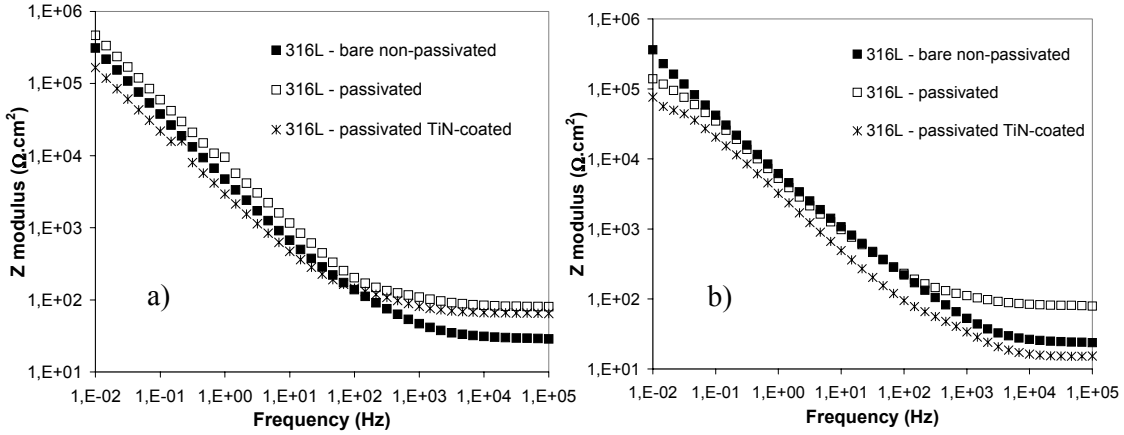

Figure 2. Bode plots (Z moduli) corresponding to: (a) 1 day and (b) 28 days of immersion in Hanks' solution at 37ºC.

The potentiodynamic polarization curves for all the materials tested after 28 days of immersion in Hanks' solution naturally aerated and at 37ºC are shown in Fig. 3.

The results of Fig. 3 show that the bare 316L specimen is prone to pitting corrosion in the Hanks' solution with a breakdown potential around +0.4V_{SCE}. However, the tendency to pitting corrosion appears at lower potentials characterized by current instabilities at potentials around +0.35V. The polarization curve of the passivated specimen shows increased corrosion current and passive current densities comparatively to the bare non-passivated one, supporting the EIS results. Although a higher breakdown potential (+0.58V) was obtained for the passivated specimen relatively to the bare one, the overpotential for passive film breakdown was similar for both specimens, bare and passivated. As mentioned previously, it is likely that the faster kinetics of oxide film growth on the 316L surface passivated might have led to a less compact passive layer. For the passivated and TiN-coated specimen, the anodic current densities obtained at low overpotentials were intermediate between the bare and passivated specimens, also confirming the EIS results. However for the TiN coated specimen, the passive range extended from +0.15 up to +1.1V. At this last potential a

sharp increase in current density occurred that could be due to the oxygen evolution reaction rather than to the onset of pitting corrosion. Paschoal et al. [16] observed pits on the surface of TiN-coated stainless steel after polarization test. This observation must be confirmed by SEM observation of the surface after polarization.

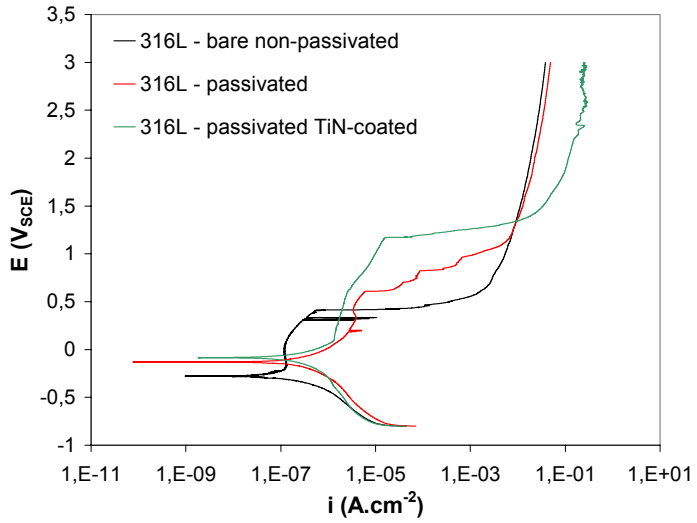

Figure 3. Potentiodynamic polarization curves after 28 days of immersion in Hanks' solution at 37ºC.

The cell viability curves of the different biomaterials investigated are shown in Fig. 4.

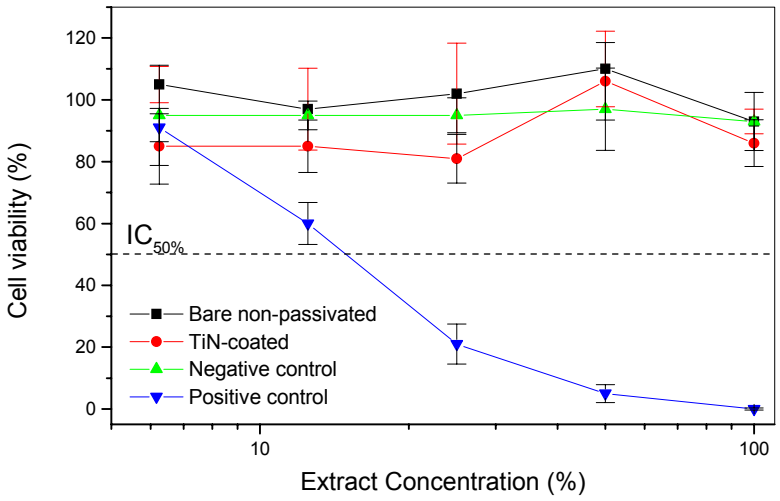

Figure 4. Cell viability curves for the different biomaterials tested.

None of the specimens tested showed any cytotoxicity, similarly to the negative control ($IC_{50\%}$ >100). To confirm the reliability of the method, the positive control showed a cytotoxic effect over the cells ($IC_{50\%}$=15). The cytotoxicity test in the biological evaluation is a toxicological screening test that can predict the biocompatibility of 316L stainless steel in the two distinct surface conditions studied. This is an indication of the suitability of TiN-coated 316L stainless steel for biomedical applications and it was an expected feature based on many literature reports on the intrinsic biocompatibility of TiN thin films [16,17].

CONCLUSIONS

The results suggest that the passive treatment used in this work must be improved to produce a more corrosion resistant substrate. However, even with the poor suitability of the passivation treatment the deposition of a TiN coating on the 316L surface markedly improved its resistance to localized corrosion. Furthermore, the non cytotoxic character of this coating is an important feature when considering its use on implant materials. A more suitable passivation pre-treatment may lead to a very stable corrosion resistance surface. Future investigations are needed to reach these objectives.

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