Susceptibility to Localized Corrosion of ASTM F745 and UNS S32750 Stainless Steels Influence of pH and Cytotoxicity Evaluation

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Abstract:

Background: Body fluids are highly corrosive as they contain chlorides and hydroxides ions, as well as salts, bacteria, proteins and dissolved oxygen. The pH of the body is usually around 7.4, although this value can vary in a range of 4 to 9 after surgery or because of haematomas, inflammations and infections. ASTM F745 (type 316L) stainless steel has been used for load bearing partial and total joint replacements and post trauma reconstructive surgeries. However, long exposure to the aggressive effect of chloride ion present in the human body, may increase the susceptibility to suffer localized corrosion. Although UNS S32750 has greater corrosion resistance to chloride ion, its magnetic characteristics inhibit its use in implantable devices. Nevertheless, this stainless steel could be used in temporary implants and orthodontic appliances such as brackets, wire arches and bands, due to its high resistance to corrosion, the greater mechanical resistance and the high capacity of plastic forming.

Objectives: The objective was to evaluate the susceptibility to localized corrosion in simulated body fluid, in the pH range of 4 to 9. Another objective was to evaluate the cytotoxicity of Cr and Ni present in the chemical deposition of both stainless steels. Cytocompatibility was also analysed by seeding cells on the surfaces of both stainless steels.

Methods: Cyclic polarization test was performed to evaluate the susceptibility to localized corrosion in 0.9 wt% NaCl aqueous solution, at pH between 4 and 9, maintained at 37°C. For cytotoxicity evaluation, neutral red, MTT and collagen assays were performed using UMR-106 cell line. Cytocompatibility was analysed by seeding UMR-106 cells on the surfaces of both stainless steels.

Results: F745-SS was more susceptible to suffer localized corrosion than UNS S32750. Although it showed a tendency to develop transpassive reactions at low pH, galvanostatic tests did not reveal the onset of localized corrosion. The results from the cytotoxicity assays indicated that no adverse effects

were observed. UMR-106 osteoblastic cells showed high viability, however, it was observed a slight reduction in the collagen production. The cytocompatibility was also satisfactory, since the cells seeded on the surfaces had adequate proliferation.

Conclusion: F745-SS is more susceptible to suffer localized corrosion than UNS S32750 in the pH range between 4 and 9. UNS S32750 showed an extensive passive region, however, transpassive reactions were observed at lower pH. On the other hand, no cytotoxic effects were promoted by both stainless steels, although a slight reduction in collagen production was observed. Cells seeded on F745-SS and UNS S32750 surfaces had an acceptable proliferation, without evidence of changes in their morphology.

Keywords: Biomaterials, stainless steels, localized corrosion, transpassive reactions cytotoxicity, cytocompatibility, proliferation.

Other

URGENT RESPONSE REQUESTED*

Dear Dr. Gregorutti,

This is with reference to the manuscript entitled "**Susceptibility to Localized Corrosion of ASTM F745 and UNS S32750 Stainless Steels. Influence of pH and Cytotoxicity Evaluation*" which has been accepted for publication in Innovations in Corrosion and Materials Science (ICMS).

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RESEARCH ARTICLE

Susceptibility to localized corrosion of ASTM F745 and UNS S32750 stainless steels. Influence of pH and cytotoxicity evaluation

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Abstract: Cyclic polarization tests assessed in 0.9 wt% NaCl solution in the pH range 4 to 9 showed that austenitic ASTM F745 is more susceptible to suffer localized corrosion than UNS S32750. Although its passivity range increased with pH (from 0.460 V to 0.600 V), its repassivation capacity diminished. In contrast, UNS S32750 showed an extensive passive region, reaching potentials close to 1.0 V/SCE. However, it has shown a tendency to develop transpassive reactions at low pH. Galvanostatic tests did not reveal the onset of localized corrosion, which would indicate that the increase in current density at potentials close to 1.0 V/SCE could be attributed to the reaction of oxygen, as well as to transpassive reactions.

The cytotoxicity was analysed by Neutral red, MTT and collagen assays, using UMR-106 osteoblastic cells. The results indicated that no adverse effects were observed on cells exposed to the extracts of both stainless steels. Neutral red and MTT assays showed high cell viability, however, it was observed a slight reduction in the collagen production. Both stainless steels showed high cell viability, however, it was observed a slight reduction in the collagen production. The cytocompatibility was also satisfactory, since the cells seeded on the surfaces had adequate proliferation, without evidence of changes in their morphology.

- **Background:** Body fluids are highly corrosive as they contain chlorides and hydroxides ions, as well as salts, bacteria, proteins and dissolved oxygen. The pH of the body is usually around 7.4, although this value can vary in a range of 4 to 9 after surgery or because of haematomas, inflammations and infections. ASTM F745 (type 316L) stainless steel has been used for load bearing partial and total joint replacements and post trauma reconstructive surgeries. However, long exposure to the aggressive effect of chloride ion present in the human body, may increase the susceptibility to suffer localized corrosion. Although UNS S32750 has greater corrosion resistance to chloride ion, its magnetic characteristics inhibit its use in implantable devices. Nevertheless, this stainless steel could be used in temporary implants and orthodontic appliances such as brackets, wire arches and bands, due to its high resistance to corrosion, the greater mechanical resistance and the high capacity of plastic forming.
- **Objective:** The objective was to evaluate the susceptibility to localized corrosion in simulated body fluid, in the pH range of 4 to 9. Another objective was to evaluate the cytotoxicity of Cr and Ni present in the chemical deposition of both stainless steels. Cytocompatibility was also analysed by seeding cells on the surfaces of both stainless steels.
- **Method:** Cyclic polarisation test was performed to evaluate the susceptibility to localized corrosion in

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0.9 wt% NaCl aqueous solution, at pH between 4 and 9, maintained at 37°C. For cytotoxicity evaluation, neutral red, MTT and collagen assays were performed using UMR-106 cell line. Cytocompatibility was analysed by seeding UMR-106 cells on the surfaces of both stainless steels.

- Results: F745-SS was more susceptible to suffer localized corrosion than UNS S32750. Although it showed a tendency to develop transpassive reactions at low pH, galvanostatic tests did not reveal the onset of localized corrosion. The results from the cytotoxicity assays indicated that no adverse effects were observed. UMR-106 osteoblastic cells showed high viability, however, it was observed a slight reduction in the collagen production. The cytocompatibility was also satisfactory, since the cells seeded on the surfaces had adequate proliferation.
- Conclusion: F745-SS is more susceptible to suffer localized corrosion than UNS S32750 in the pH range between 4 and 9. UNS S32750 showed an extensive passive region, however, transpassive reactions were observed at lower pH. On the other hand, no cytotoxic effects were promoted by both stainless steels, although a slight reduction in collagen production was observed. Cells seeded on F745-SS and UNS S32750 surfaces had an acceptable proliferation, without evidence of changes in their morphology.

Keywords: Biomaterials, stainless steels, localized corrosion, transpassive reactions cytotoxicity, cytocompatibility.

1. INTRODUCTION

The main properties that the materials used in biomedical applications must satisfy are corrosion resistance and biocompatibility. Body fluids are highly corrosive as they contain chlorides and hydroxides ions, as well as salts, bacteria, proteins and dissolved oxygen. The pH of the body is usually around 7.4, although this value can vary in a range of 4 to 9 after surgery or because of haematomas, inflammations and infections [1-5]. These changes can increase the risk of corrosion and consequently, the release of metallic ions, which may cause cytotoxic effects in the body tissue [6-8]. At the same time, the biocompatibility is related to the cytotoxicity effects of the alloy components and the damage they can cause to the cells during the prolonged time in which the prosthesis is implanted in the body. In the biological response of the organism to the presence of any implant, it is important to consider the capacity of osteoblasts and fibroblast proliferation on the metallic surface to ensure the osseointegration to the bone [9,10].

One of the materials widely used for load bearing partial and total joint replacements and post trauma reconstructive surgeries is the austenitic stainless steel 316L, which can be used to elaborate forged and machined devices (specification ASTM F138/139) or for cast implants (specification ASTM F745, hereafter F745-SS). These alloys have satisfactory performance as biomaterial, in addition to their low cost compared to Ti and Co-Cr-Mo alloys [11]. However, long exposure to the aggressive effect of Cl ion present in the human body, may increase the susceptibility to suffer localized corrosion [1, 12-13]. On the other hand, UNS S32750 is a dual phase austenite/ferrite stainless steel (duplex stainless steel), which has greater corrosion resistance to chloride ion, reason by which it could be considered as alternative to 316L, but it has not yet been accepted as biomaterial [14,15]. Although some authors have carried out in vivo and in vitro studies aimed at orthopedic applications, its magnetic characteristics inhibit the use in implantable devices [16-19,20]. However, this stainless steel could be considered as an alternative for temporary implants such as plates and screws, in which pitting and crevice corrosion could occur [1]. Another possible application would be in orthodontics as has been reported by other authors [21-23], in which the use is also temporary. UNS S32750 could be appropriate for making orthodontic appliances such as brackets, wire arches and bands, not only to its high resistance to corrosion, but also for the greater mechanical resistance and the high capacity of plastic forming.

F745-SS and UNS S32750 have Cr and Ni in their chemical composition, elements which may cause different systemic-toxic reactions. In particular, Ni is known as allergenic agent and has some carcinogenic potential [24-31]. Considering the possible changes in pH that can occur in the human organism, either in the oral or corporeal environment, the present work evaluates the resistance to localized corrosion of the mentioned stainless steels in the pH range between 4 to 9. At the same time, the possible cytotoxic effect of Cr and Ni was analysed by Neutral red, MTT and collagen bioassays.

2. MATERIALS AND METHOD

2.1 Materials

F745-SS and UNS S32750 were obtained by means of investment casting process (IC), which is one of the techniques used to manufacture surgical implants [11]. In order to perform the analysis with similar microstructures of cast prosthesis, melting was carried out in a medium frequency induction furnace. The casting temperature was 1600 °C, and following the usual practice of the IC process, the shell mould was heated up to 800 °C to increase its permeability and reduce the thermal gradient of cooling, in order to improve castability. The final chemical compositions in wt.% were as follows:

0.04 %C, 18.09% Cr, 12.46% Ni, 2.02% Mo, 0.49% Si, 0.09% Mn for F745-SS; 0.03 %C, 25.22% Cr, 7.07% Ni, 4.10% Mo, 1.00% Si, 0.78% Mn, 0.30%N, for UNS S32750.

Both alloys were solution-annealed to dissolve undesired phases that usually precipitate during solidification of cast stainless steels. F745-SS was heated at 1080 °C during 2 hours and water quenched, in order to eliminate the δ -ferrite present in the as cast structure, while UNS S32750 was heated to 1120 °C for 1 hour, then cooled in furnace down to 1045 °C and maintained for 1 hour, and subsequently water quenched, to dissolve the sigma phase.

X-ray diffraction was performed to identify the phases present in both stainless steels after the solubilized treatment, using a 3050 Philips goniometer and CuK α radiation. The divergent slit was 1° and the scanning conditions were steps of 0.02° with a counting time of 1 second per step, being 2 θ between 30 and 100°.

2.2 Electrochemical tests

The susceptibility to localized corrosion was evaluated by cyclic polarization tests. The electrochemical measurements were carried out at 37° C using a conventional electrochemical cell with a three-electrodes arrangement. The working electrode was a cylinder sample (ϕ = 6 mm and length = 20 mm) polished at #600 by silicon carbide paper. The counter and reference electrodes were respectively, a stainless steel cylinder coaxial to the sample and a Saturated Calomel Electrode (SCE). Cyclic polarization was performed with the EG&G Potentiostat/Galvanostat Model 273A controlled by CorrWare® program. The samples were kept for 1 hour at the open circuit potential (E_{oc}). The potential scan was performed in the anodic direction at 0.167 mVs⁻¹ from a potential value 0.20 V more cathodic than E_{oc} up to reach the reversal conditions (E = 3V/SCE or j = 10⁻⁴ A cm⁻²). To simulate the human environment, a 0.9 wt% NaCl (NaCl 99+%, A.C.S.) aqueous solution was used as electrolyte. The pH was varied between 4 and 9 by addition of NaOH or HCl solution. Galvanostatic tests were performed on UNS S32750 to assess the possible interference of the oxygen reaction. The measurements were at a constant current of 1 10⁻⁴ A for 3 hours, using 0.9 wt% NaCl solutions at pH 4, 7 and 9. In addition, particular tests were performed at pH 9, with currents of 6.2 10⁻⁶ A and 1.2 10⁻⁵ A.

2.3 Cytotoxicity

UMR-106 cell line was originally obtained from American Type Culture Collection (ATCC). The cells were grown in Falcon T-25 flasks, maintained at 310K (37°C) in a humid atmosphere with 5% CO₂. As culture medium (CM), 10 mL DMEM high glucose supplemented with 10% inactivated fetal calf serum, 100 IU mL⁻¹ penicillin and 100 µg mL⁻¹ streptomycin sulfate was used.

Extraction vehicles (EV) were obtained exposing the CM to F745-SS and UNS S32750 as follows: area of alloy/volume of CM ratio of 2 cm² mL⁻¹, incubated at 37°C for 3 days (72 h) according to ISO 10993-5 Standard. Their effects on the cells were compared with a negative control (-Control), consisting of the CM not exposed to the alloys.

Neutral red (NR) assay determines the cell viability using a NR dye (3-amino-7-dimethyl-amino-2-methyl-phenazine hydrochloride), which is incorporated and accumulated in the endosomes and lysosomes. The NR dye absorbed is proportional to the number of viable cells, indicating the good integrity of the plasmatic membrane. For the test, 1.5 10⁴ UMR-106 cells/well were cultivated overnight in 96-well tissue culture plate with the CM previously described, at 37 °C in a humid atmosphere with 5% CO₂. The CM was subsequently replaced by each EV and -Control, and incubated again for 24 hours at the same conditions. After that, NR dye was added and incubated for 3 hours. Finally, the cells were washed with PBS and NR dye was solubilised in a mixture of water/ethanol/acetic acid dye for the absorbance measurement by a spectrophotometer at 570 nm.

MTT evaluates the cell proliferation by soluble yellow MTT tetrazolium (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) reduction to insoluble purple formazan by the action of succinate dehydrogenase enzyme present in mitochondria.

The test was performed on 1.5 10⁴ UMR-106 cells, following the same procedure as NR assay. In this case, 5 mg mL⁻¹ of dye MTT was added and the obtained formazan was solubilised with DMSO. The absorbance was measured at 570 nm.

For collagen assay, 8.0 10³ UMR-106 cells/well were cultivated overnight in 96-well tissue culture plate, at 37 °C in a 5 % CO₂ atmosphere. The cells were then exposed to both EV and the -Control for 7 days (168 hours). After that, the cells were fixed with a mixture of formaldehyde and picric acid for 1 hour at room temperature. Sirius red dye solution was added and again incubated at room temperature for 1 hour. A Subsequent washing with 0.01N HCl solution was made to remove the unincorporated dye in collagen. To extract the incorporated dye into the molecules of collagen, a NaOH solution was added. The absorbance was measured at 550 nm.

Three experiments were carried out by assay, performing eight measurements in each case. The results were relative to the -Control, having been considered that the average of the optical density of the -Controls was 100%. The data were analysed by Student's t-test, considering the statistical significance at a probability $p < 0.05$.

For cytocompatibility, UMR-106 cells were directly seeded onto the surface of F745-SS and UNS S32750 to evaluate their proliferation after 3 days. Cells were stained with acridine orange and ethidium bromide to be observed by fluorescence microscopy.

3. RESULTS

3.1 Microstructural analysis

In a previous work (20), the microstructures of both stainless steels, which depend on the balance of the stabilizing elements of austenite and ferrite, were analyzed.

F745-SS has fully austenitic structure because of the Ni content between 12% and 14%. Figure 1 (a) shows the microstructure after solution-annealing heat treatment, without δ -ferrite that usually precipitates in the interdendritic regions because of the microsegregation of Cr and Mo during solidification. At the same time, the solidification cells can be seen, with the evidence of the dendritic structure inside. In contrast, UNS S32750 has a ferrite-austenite structure in an approximate fifty-fifty proportion, as shown in Figure 1 (b). The microstructure is the result of the higher content of Cr (24.0 – 26.0 wt.%) and Mo (3.0 – 5.0), and the lower amount of Ni (6.0 – 8.0 wt.%). Sigma phase, which usually precipitates in this stainless steel at the ferrite/austenite interface, in the range between 830 °C and 470 °C during cooling after solidification, has been dissolved in the solution-annealing treatment.

Figure 1. Microstructures after solution-annealing treatment, revealed using 3 parts HCl, 1 part HNO₃, 1 part acetic acid; (a) ASM F745-SS; (b) UNS S32750.

The present phases were identified by X-ray diffraction, as shown in Figure 2.

The diffractogram corresponding to F745-SS shows the characteristic peak (111) of austenite and minor peaks of the planes (200), (220) and (222). Diffractions of the ferrite phase have not been observed.

In the case of UNS S32750, in addition to the aforementioned peaks of austenite, the diffractions of the planes (110), (200), (211) and (220) of the ferrite are observed.

Figure 2. Diffraction patterns using CuK α radiation; (a) F745-SS and (b) UNS S32750.

3.2 Cyclic polarization test

Cyclic polarization test determines three significant potentials: E_{Corr} , potential at the open circuit condition; E_{Pit} , potential at which the passivity is broken and E_b , protection potential at which the hysteresis loop is completed upon reverse polarization scan. The greater the difference ($E_{\text{Pit}} - E_{\text{Corr}}$) the more passive the material is, and consequently, less prone to suffer localized corrosion. At the same time, the lesser difference ($E_{\text{Pit}} - E_b$) indicates a higher tendency to repassivation. The tests were performed using NaCl solution with Cl^- concentrations in the range of 5790 - 5880 mg L⁻¹. The choice of this solution was because its Cl concentration is higher than other solutions such as Ringer's, Hank's or artificial saliva, so its aggressiveness from the point of view of corrosion is greater.

Figures 3 to 5 show the electrochemical behavior of F745-SS and UNS S32750 in the pH range 4 to 9.

Figure 3. Cyclic polarization curves in 0.9 wt% NaCl aqueous solution at pH 4 and 5, and 37 °C, (D-SS indicates UNS S32750).

The polarization curves corresponding to F745-SS evidenced shorter passivity range than those of UNS S32750, with fluctuating current signal, which could be related to the instability of the Cr_2O_3 protective layer. At the same time, the wide hysteresis loop indicates its low capacity to repassivate. The electrochemical performance was greatly influenced by the pH variation, while E_{Corr} and E_{Pit} potentials shifted in general to more electropositive values, E_b decreased at increasing pH.

Figure 4. Cyclic polarization curves in 0.9 wt% NaCl aqueous solution at pH 6 and 7, and 37 °C.

Figure 5. Cyclic polarization curves in 0.9 wt% NaCl aqueous solution at pH 8 and 9, and 37 °C.

According to those potentials, F745-SS exhibited greater passivity region as pH increases, varying from 0.463 V/SCE (pH 4) to 0.605 V/SCE (pH 9), but contrarily, its repassivation capacity decreased from 0.548 V/SCE (pH 9) to 0.130 V/SCE (pH 4).

At pH 4 and 5, the current density increased markedly at potentials close to -0.200 V/SCE, before the passivity period. That increment was not observed at higher pH.

Table 2 reports the values of the potentials recorded in the cyclical polarization tests, whose evolution with respect to pH is shown in Figure 6.

Table 2. Potentials obtained from cyclic polarization tests of F745-SS.

pH	E_{Corr} V/ (SCE)	E_{pit} V/ (SCE)	E_b V/ (SCE)	$E_{pit} - E_{Corr}$ V	$E_{pit} - E_b$ V
4	-0.343	0.120	-0.010	0.463	0.130
5	-0.281	0.282	-0.035	0.563	0.317
6	-0.249	0.349	-0.070	0.598	0.419
7	-0.260	0.314	-0.108	0.574	0.422
8	-0.227	0.280	-0.165	0.507	0.445
9	-0.216	0.389	-0.159	0.605	0.548

On the other hand, UNS S32750 showed, for all the considered pH, a more extended passivity range, observing an increase of the current density from ~1.0 V/SCE, and ending with a narrow hysteresis loop. At lowest pHs (4 and 5) an anodic current peak, probably related with a transpassive reaction, was observed at potentials close to 0.845 V/SCE. This event tended to disappear as the pH increased.

Another peculiarity to be recorded corresponds to pH 9 and is presented at potentials near to 0.82 V/SCE and 1.10 V/SCE, at which two small peaks were observed. Considering the moderate slope of increasing current density and the close hysteresis loop, they would be linked to transpassive reactions.

For all the pH, the current density increased from potentials close to 1.0 V/SCE, reason by which that increase could not only be attributed to the breakdown of passivity, but also to the possible oxygen reaction. In order to evaluate this possibility, galvanostatic tests were performed at pH 4, 7 and 9, applying a current step ($j = 1 \cdot 10^{-4} \text{ A cm}^{-2}$) equivalent to the reversal condition defined for cyclic polarization. For comparison, F745-SS at pH 7 was also tested.

Figure 6. Evolution of corrosion potential (E_{Corr}), pitting potential (E_{Pit}), protection potential (E_b), passivity range ($E_{Pit} - E_{Corr}$) and repassivation capacity ($E_{Pit} - E_b$) with pH, corresponding to F745-SS.

Figure 7 shows that for all the pH considered, the potential increased to a maximum potential close to 1.0 - 1.1 V/SCE, and subsequently decreased, stabilizing to a potential corresponding to the passive region.

Figure 7. Galvanostatic curves of UNS S32750 in 0.9 wt% NaCl aqueous solution, at pH 4, 7 and 9, and 37 °C. The curve corresponding to F745-SS was performed in the same solution and temperature, at pH 7.

The potential evolution for pH 4 also show a shoulder at $E \approx 0.8$ V/SCE, in accordance with the anodic peak recorded in Figure 3.

The potential evolution for F745-SS was highly fluctuating, decreasing toward less electropositive values, up to near the repassivation potential (≈ 0.06 V/SCE) observed in the respective polarization curve. That fluctuating response would indicate the onset of localized corrosion.

The final surface condition for both materials after the galvanostatic tests are shown in Figure 8. While corrosion pits were noticed in F745-SS, no evidence of localized corrosion was observed in UNS S32750.

Figure 8. Micrographs taken after galvanostatic tests obtained by SEM at 5000X. (a) Pitting damage on F745-SS; (b) Surface of UNS S32750 without evidence of pitting.

The analysis of the distribution of sizes of the pits shown in Figure 9, indicated that the greatest amount is in the range between 0.38 μm and 0.54 μm , the general average being 0.49 μm .

Figure 9. Distribution of pit sizes, made on fifty measurements in an area of 240 μm^2 .

3.3 Cytotoxicity

Although UNS S32750 is not used for surgical implants, it was used as a reference with respect to F745-SS, because it contains a higher percentage of Cr but less Ni. However, the possible release of ions in the oral environment should also be considered if its use in orthodontics is contemplated.

The possible cytotoxic effect of both stainless steels was evaluated by NR, MTT and collagen assays using UMR-106 osteoblast-like cells, because this cell line has characteristics of bone cells. At the same time, the cytocompatibility was evaluated by direct contact method, seeding UMR-106 cells onto the surfaces.

NR assay evaluates lost in cell viability, when a determined element or compound affects the integrity of the plasma membrane. The cells that do not suffer alterations in the plasma membrane can endocitate correctly the dye that is accumulated in lysosomes and endosomes. This phenomenon evidences the amount of living cells present in the CM exposed to a toxic agent [32,33].

MTT assay evaluates the cellular mitochondrial activity. When MTT enters the cells and passes into the mitochondria it is reduced to an insoluble formazan by the succinate dehydrogenase enzyme. Cells with low metabolism reduce very little MTT, while rapidly dividing cells exhibit high MTT reduction rate.

The results of NR and MTT assays are shown in Figures 10 and 11, in which it is observed that the cells exposed to EV-F745SS and EV-UNS showed high cell viability.

Figure 10. Viability of UMR-106 cells after the exposition to EV-F745SS and EV-UNS in NR assay. Influence of F745-SS and UNS S32750 on endosomes and lysosomes. The -Control is the medium not exposed to the stainless steels.

Figure 11. Viability of UMR-106 cells after the exposition to EV-F745SS and EV-UNS in MTT assay. Influence of F745-SS and UNS S32750 on mitochondrial metabolism. The -Control is the medium not exposed to the stainless steels.

Although the absorbance values in NR were slightly lower than -Control, in the MTT they were close to this reference parameter, inferring that F745-SS and UNS S32750 did not promote adverse effects in the osteoblastic cells.

Collagen is the protein that constitutes 85% of the extra cellular matrix of bone and has an important role in the differentiation and function of osteoblasts that participate in the implant osseointegration [10,34,35]. The test consists of coloring the collagen produced by the cells of osteoblastic origin with sirius red dye.

Cells exposed to EV-F745SS and EV-UNS extracts, obtained after 7 days in contact with both stainless steels, experienced a slight reduction in collagen production with a decrease of the statistical significance ($p < 0.05$) respect to -Control, as shown in Figure 12.

Figure 12. Collagen production of UMR-106 cells after the exposition to EV-F745SS and EV-UNS. The -Control is the medium not exposed to the stainless steels. * Statistical significance $p < 0.05$.

To evaluate the cytocompatibility of the stainless steels, direct contact method was performed seeding UMR-106 cells on their surfaces. The cells were also seeded in a polystyrene plate used as a reference, to perform a qualitative analysis on the effect of both materials on cell development.

The images in Figure 13 show that the cells adhered and proliferated properly on the materials, compared to the inert polystyrene plate. Furthermore, no changes in cell morphology have been noted, so it is conjectured that the materials do not cause adverse effects on direct contact with cells.

Figure 13. Images of UMR-106 cells seeded on both stainless steels after three days: (a) F745-SS, (b) UNS S32750, (c) Polystyrene standard culture plate used as reference.

4. DISCUSSION

4.1 Cyclic polarization test

As it has been previously mentioned, F745-SS and UNS S32750 differ in chemical composition and microstructure. Nilsson [36,37] in his study on the critical pitting temperature of austenitic and duplex stainless steels, concluding that the resistance to localized corrosion depends on the chemical composition rather than on the microstructure. The pitting resistance equivalent number (PREN) empirically represents the effect of Cr, Mo and N on corrosion resistance, as follows:

$$\text{PREN} = \text{wt.\% Cr} + 3.3 \text{ wt.\% Mo} + 16 \text{ wt.\% N}$$

According to chemical compositions, the greater amount of Cr and Mo, and the N content of UNS S32750 determines its highest PREN compared to the F745-SS, 43.5 and 24.8, respectively, and consequently its better performance to localized corrosion.

Similarly, other authors have referred to the synergistic effect of Cr and Mo in the passive film of stainless steels and the strong improvement that Mo and N produces in the resistance to corrosion localized to the attack of chloride ions [12,38-40].

In the potentiodynamic response of the F745-SS, the current peak prior to the passive zone ($E \sim -0.200$ V/SCE) observed at low pH can be related to the active-passive transition, also described Kim and Young in their study of passive film of 316L stainless steel [41]. That transition defines a critical current that must be overcome in order for the passive film to form, as mentioned by Shoensmith [42].

On the other hand, E_{corr} showed a shift towards more electropositive potentials at increasing pH as well as E_{pit} , although in this case a slight decrease was observed between pH 7 and 8, it follows an increasing trend, as seen in Figure 6. This results agrees with those obtained by Malik et al. [43], who studied the influence of chloride concentration and pH on the pitting behaviour of AISI 316L in aqueous solutions. Although the concentration of Cl^- in the solutions used in the present work was slightly higher than the reference paper has, the trends shown by the electrochemical potentials were similar.

In Figure 6, it was also observed that the passivity range showed a slight increasing trend with pH, despite the slight decrease registered at pH 8. Similarly, the repassivation capacity was increasing with pH, although the implications are opposite in each case. The greater difference ($E_{\text{pit}} - E_{\text{corr}}$) indicates a lower susceptibility to localized corrosion, while the increase in difference ($E_{\text{pit}} - E_b$) implies that the F745-SS has a lower capacity to repassivate in the event of a pitting.

Consequently, possible alterations in pH that could occur in the human body could negatively influence the electrochemical stability of F745-SS, since a decrease would imply a more unfavorable situation regarding its response to corrosion.

In the case of UNS S32750, the transpassive reactions observed in the polarization curves can be attributed to its higher Cr content. Transpassive reactions in high Cr stainless steels involves the oxidation of Cr^{+3} to Cr^{+6} leading to the formation of soluble ions as $\text{Cr}_2\text{O}_7^{2-}$ or CrO_4^{2-} . This reaction was more noticeable at low pH, while as pH increased, the potential as well as the current of the transpassive oxidation decrease. The current peak shifted from -0.845 V/SCE at pH 4 to -0.75 V/SCE at pH 7, value that matches with those reported by Schmucki et al. [44] in metallic Cr, and Pound [38], Hodgson et al. [45], and Kociubczyk et al. [46], in CoCrMo biomaterial. Transpassive reactions in UNS S32750 were also observed in NaOH alkaline solutions, as mentioned by Chan and Tjong [36] in their review on the effect of secondary phases on the corrosion behavior of this stainless steel.

Although the test conditions differ from those in the Pourbaix diagram, it could be used as a thermodynamic reference to analyze the feasibility of the different redox reactions that UNS S32750 would suffer because of the pH variation. Then, from the Pourbaix diagram of Cr shown in Figure 14, it could be inferred that at low pHs the oxidation of Cr^{+3} to Cr^{+6} would lead to the formation of $\text{Cr}_2\text{O}_7^{2-}$, while from pH 7 CrO_4^{2-} would form [43]. In this paper, it was also mentioned that at first instance the CrO_4^{2-} is trapped in the Cr_2O_3 film prior to transpassive dissolution, these events occurring at two different potentials.

That mechanism of entrapment of CrO_4^{2-} and subsequent dissolution could explain the potential step observed in the polarization curve at pH 9.

Another feature to be considered is the possible occurrence of the oxygen reaction, taking into account the potentials reached by the UNS S32750 in the polarization curves. For the whole range of pH analyzed, the galvanostatic tests showed that the potential remained in an extended plateau over time, whose value corresponds to potentials within the passivity zone of UNS S32750 recorded in the cyclic polarization curves. The stability of the potential indicates that localized corrosion did not occur, hence the increases in the current density in the polarization curves could correspond to the oxygen reaction. Pan et. al [16], in their electrochemical study on stainless steels for biomedical application referred to the probable oxygen evolution at the polarization level closed to 1.0 V/SCE, while Kang and Lee [47] mentioned that the higher Cr content in UNS S32750 increases the passivity range up to potentials at which the oxygen evolution could take place.

Figure 14. Pourbaix diagram of Cr.

4.2 Cytotoxicity

From the high cell viability observed in NR and MTT assays, it could be argued that the extracts exposed to F745-SS and UNS S32750 for 3 days did not cause alterations in the plasma membrane, nor adverse effects on mitochondrial activity of UMR-106 osteoblastic cells. Differences in chemical composition and microstructure did not produce significant differences in the bioassays performed, observing that the results were within the considered statistical significance respect to -Control ($p < 0.05$).

In vitro studies performed by other authors on such stainless steels, but using different cell lines, yielded similar results. Sumarta et al. [48], showed the good biocompatibility of AISI 316L to fibroblast cells from BHK-21 cell line in maxillofacial reconstruction plates, while Wang et al. [33] also refer to the no adverse effects of AISI 316L on cell viability of L929 and NIH3T3 cells. Beloti et al. [18] studied the behavior of a duplex stainless steel, in addition to austenitic ASTM F138, using rat bone marrow cells, concluding that cell viability was not affected by the chemical composition of the materials or by the duration of the culture.

On the contrary, it was observed a slight adverse effect on the collagen production for both stainless steel. However, considering that no toxic effects were observed in NR and MTT assays, the cause of the decrease in collagen production could not be determined.

On the other hand, the achievement of a good osseointegration requires the satisfactory adhesion and proliferation of osteoblastic cells on the surface of the biomaterial. The UMR-106 osteoblastic cells seeded directly on the surfaces of F745-SS and UNS S32750 showed a good anchorage, proliferating in an acceptable manner and maintaining their morphological characteristics. The chemical composition of the surface is one of the features that could affect cell proliferation, and hence, the osseointegration of the biomaterial. In a previous work, Talha et al. [49] in their cytocompatibility study of 316L and Ni free steels using another cell line (DL cell line), concluded that the absence of Ni and the increase of N benefit cell proliferation. In the present case, the qualitative analysis performed showed no differences in cell proliferation, despite the different concentrations of Cr, Ni, Mo and N of both stainless steels. It could be argued that a surface layer composed mainly of chromium oxide (Cr_2O_3), so the differences in the respective nominal chemical compositions would not influence cell adhesion covers F745-SS and the UNS S32750.

5. Conclusions

In the considered experimental conditions, F745-SS is more susceptible to suffer localized corrosion than UNS S32750. The particular analysis of the electrochemical response shown that the passivity range of F745-SS increased with pH, meanwhile the passivity current as well as its repassivation capacity diminished. On the other hand, UNS S32750 showed an extensive passive region, up to potentials close to 1.0 V/SCE, however, transpassive reactions were observed at lower pH. Galvanostatic tests did not reveal the onset of localized corrosion, which would indicate that the increase in current density at the potentials close to 1.0 V/SCE could be attributed to the oxygen reaction, as well as to transpassive reactions.

From the biocompatibility point of view, NR and MTT assays showed satisfactory cell viability for both stainless steels. On the other hand, F745-SS and UNS S32750 would promote a slight adverse effect in collagen production. Although this reduction would not be attributable to cytotoxic effects, according to the results obtained in the RN and MTT tests.

Cytocompatibility assay showed that the cells seeded on the F745-SS and UNS S32750 had an acceptable proliferation, without evidence of changes in their morphology.

Accordingly, both stainless steels act as appropriate scaffolding for the cells.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

This document does not contain any studies with humans or animals.

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