New approaches regarding the electrochemical behaviour of the Ti-6Al-4V alloy functionalized with antimicrobial peptide JI-2 via plasma polymerization

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Research Article

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Abstract

Ti-6Al-4V alloy is widely used in the biomedical sector due to its excellent biocompatibility, corrosion resistance, and mechanical properties. However, the use of this material as an implant is still susceptible to failure due to bacterial adhesion on the surface. Initial studies performed by this research group demonstrated and characterized the functionalization process of the Ti-6Al-4V alloy with antimicrobial JI-2 peptide via plasma polymerization method. However, the electrochemical properties of the functionalized system are still little explored. Here, we present new insights regarding the global electrochemical behaviour of the functionalized Ti-6Al-4V with PAA/JI-2 in 0.6 mol L\(^{-1}\) NaCl solution. Open circuit potential and electrochemical impedance spectroscopy techniques were used. The electrochemical tests were accompanied by a detailed microstructural analysis by SEM. The results demonstrated the PECVD technique was advantageous to produce thin films based on acrylic acid monomer on the Ti-6Al-4V surfaces. The PAA/JI-2 thin films shifted the corrosion potential (\(E_{\text{corr}}\)) of the Ti-6Al-4V alloy to more positive values, indicating a better thermodynamic stability. The capacitance of the barrier layer decreases for the uncoated specimens with increasing immersion time, suggesting an increase in the thickness of the native oxide layer. In contrast, the opposite effect was verified for the Ti-6Al-4V/PAA/JI-2.

1. Introduction

Titanium and titanium-based alloys are biomaterials frequently used in biomedical applications, such as orthopedic [1], dental [2], and artificial corneal implants [3]. Among titanium alloys, the Ti-6Al-4V stands out due to its excellent mechanical properties, biocompatibility, high resistance to corrosive processes as well as low Young’s modulus when compared to other metallic biomaterials [4–6]. The good corrosive properties of the Ti-6Al-4V alloy may be attributed to the TiO\(_2\) thin film (4–6 nm of thickness) that spontaneously forms on the material surfaces [7]. As reported by Godoy-Gallardo et al [2], titanium-based implants are susceptible to the risk of biological failure promoted by the formation of bacterial biofilm.

Surface modifications in metallic biomaterials are able to improve their osseointegration properties [8], antimicrobial and antibiofilm activities [9–10], endothelialization [11] and long-term stability in the physiological environment [12]. Among the numerous strategies for surface treatment, there is the functionalization of biomaterials with bioactive molecules in which the use of antimicrobial peptides (AMPs) has been shown to be quite effective [2, 9–10, 13–14]. AMPs are small cationic and amphipathic molecules that have inhibitory action against a broad spectrum of gram-positive and gram-negative bacteria [2, 15]. Jelleines constitute a family of AMPs extracted from royal jelly, an acidic secretion produced by worker bees [16]. The study of the biological and biophysical properties of this family led to the development of new peptides with amino acid sequences analogue to Jelleine-I, which prove to be potential antimicrobial agents [17–18].

Recently, our research group succeeded in immobilizing a new analogue of Jelleine-I named JIChis-2 (JI-2) on Ti-6Al-4V alloy [19]. For this purpose, plasma technique was used to carry out the polymerization of acrylic acid. This provided the formation of a thin film of polyacrylic acid (PAA) on the alloy surface, which can be used as a precursor to immobilize molecules due to the presence of \(-\text{COOH}\) groups [20–21]. In fact, FTIR results showed that N terminus of JI-2 was covalently bound to the material’s surface after activation of the carboxyl groups with 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide and N-hydroxysuccinimide (EDC/NHS). This type of immobilization is an irreversible coupling that ensures greater stability of the peptide on the metallic surface compared to reversible ones [2, 14, 22]. Despite this, initial studies on the immersion and localized electrochemical behaviour of the new material showed the need for a more in-depth study on this stability, especially when considering the aggressiveness of the physiological environment [19]. It is important to point out that although there are a large number of studies that perform the immobilization of bioactive peptides in metallic alloys, very few explore the effects of this treatment at the electrochemical level [2, 9–10, 13–14, 23].

Considering this scenario and the fact that understanding the corrosion processes of metallic materials is a crucial factor in biomedical devices, since the release of metallic ions in the body is associated with the development of several diseases [24]. Here, we present an electrochemical study of the new material in search to explore its stability under aggressive conditions. For this, the characterization of the electrochemical behaviour of Ti-6Al-4V functionalized and non-functionalized with PAA and peptide JI-2 in 0.6 mol L\(^{-1}\) NaCl solution was performed. Then, open circuit potential (OCP) and electrochemical impedance spectroscopy (EIS) techniques were carried out accompanied by detailed analyzes of the microstructure of the samples by scanning electronic microscopy. To our knowledge, this is the first work that considers the electrochemical behaviour of the Ti-6Al-4V alloy functionalized with antimicrobial peptide JI-2 via plasma polymerization.

2. Experimental

2.1 Materials

Ti-6Al-4V sheets with dimensions of 1 cm x 1 cm, JI-2 antimicrobial peptide provided by GenScript, 99% Acrylic Acid (AA) from TCI América, Inc., 99% N-hydroxysuccinimide (NHS) from Oakwood Chemical, 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) from Oakwood Chemical, Phosphate-Buffered Saline (PBS) and sodium chloride (NaCl).

2.2 Preparation and functionalization of the Ti-6Al-4V specimens

Before the global electrochemical tests, the Ti-6Al-4V substrates were sanded with silicon carbide (SiC) sandpaper in the sequence of 800, 1200, 2500 and 4000#. Then, the materials were polished with a 3 µm diamond paste. After the mentioned processes, the samples were washed with detergent, distilled water, and isopropyl alcohol in ultrasound. The cleaning procedure lasted 10 min for each medium. The method of acrylic acid deposition and immobilization of the JI-2 peptide was previously described by this group and further information can be found in reference [19], using a custom design microwave-assisted PECVD system. Afterwards, Ti-6Al-4V specimens containing PAA thin films were immersed in a carboxyl group activation solution EDC/NHS (1:1) in 10 mmol L\(^{-1}\) for.
a period of 24 h. Finally, the functionalized specimens were immersed in a peptide solution at a concentration of 80 µmol L\(^{-1}\) during 36 h. Thus, the surfaces were covalently immobilized through the interaction of the carboxyl groups of the PAA-containing surface and the amine group of the peptide.

### 2.2 Electrochemical Tests

In the present work, three electrochemical tests were used to evaluate the influence of the surface treatment on the Ti-6Al-4V alloy. For this purpose, a conventional electrochemical cell containing the compartment of three electrodes was used: working electrodes (WE) composed of Ti-6Al-4V and Ti-6Al-4V/PAA/JI-2 specimens with a useful area of 1 cm\(^2\), a platinum wire as a counter electrode (CE) and saturated calomel electrode (Hg/Hg\(_2\)Cl\(_2\), KCl\(_{sat}\)) as reference electrode (RE). All electrochemical tests were performed using a 0.6 mol L\(^{-1}\) NaCl solution and a potentiostat/galvanostat (µStat-i-400s) at room temperature. Although the body fluid has a concentration of approximately 0.1 mol L\(^{-1}\) NaCl, in the present work it was decided to use a more concentrated solution to guarantee the efficiency of the performed treatments. All electrochemical tests were replicated and showed good reproducibility. In the present work, the OCP tests were carried out with the aim of verifying the thermodynamic stability of the Ti-6Al-4V and Ti-6Al-4V/PAA/JI-2 specimens in a medium containing aggressive ions. The corrosion potential (\(E_{corr}\)) of the samples was monitored during 3,600 s. The EIS spectra were obtained for all specimens in the frequency range of 100 kHz to 10 mHz using an applied a.c. signal of 10 mV (rms) in order to guarantee linearity of the electrode response. The EIS spectra were monitored every hour over a period of 12 h and then measurements were taken at 24 h and 168 h, respectively. For EIS data fitting, the ZView2 software was used. It is important to mention that before each EIS measurement, the OCP parameter was evaluated during 600 s.

### 2.3 Morphological and structural characterization

In the present work, the morphology and composition of the Ti-6Al-4V and Ti-6Al-4V/PAA/JI-2 specimens were assessed by scanning electron microscopy (SEM) before and after the EIS tests. In this sense, an analytical FE-SEM JEOL 7001F equipped with an Oxford light elements EDS detector was used at the Chemistry Department of the Federal University of Uberlândia, Minas Gerais state, Brazil. To obtain SEM images with a high resolution, all the samples containing PAA/JI-2 compounds were sputtered by Au element.

### 3. Results & Discussion

The corrosion behaviour of the Ti-6Al-4V and Ti-6Al-4V/PAA/JI-2 substrates was investigated by global electrochemical techniques. In view of this, the OCP technique is a crucial parameter for understanding the corrosion tendency of metallic materials [25]. In short, OCP is a non-destructive technique obtained by the potential difference between the metallic surface under study (working electrode) and the reference electrode [26]. Representative curves of the OCP evolution with the immersion time for the Ti-6Al-4V and Ti-6Al-4V/PAA/JI-2 samples are presented in Fig. 1. Considering the non-functionalized Ti-6Al-4V substrate, the OCP stabilized at approximately ~ 0.24 V. Mahlobo et al. [27] evaluated the corrosion resistance of the Ti–6Al–4V alloy fabricated via powder rolling technique under various simulated human body solutions (0.6 mol L\(^{-1}\) NaCl, Hank's balanced salt solution (HBSS) as well as Ringer's solution. The results obtained by Mahlobo & collaborators are in line with our results (-0.26 V vs. Ag/AgCl) compared to the (-0.24 V vs. Hg/Hg\(_2\)Cl\(_2\), KCl\(_{sat}\)). In contrast, surfaces containing the JI-2 peptide were more stable since the beginning of the immersion reaching approximately ~ 0.03 V after 700 s. The same behaviour was found by Trino and collaborators [23] which compared the OCP tests of pristine Ti and DMP1 peptides-Ti. From this, it is possible to conclude that the process of inserting the peptide into the system shifted the potential to slightly negative values. By showing that the functionalized material, at this first moment, displays a better thermodynamic stability when compared to the base material.

Electrochemical Impedance Spectroscopy (EIS) is an important non-destructive technique for the characterization of electrochemical systems [28–29]. In this perspective, this technique was adopted to understand the corrosion behaviour of the Ti-6Al-4V and Ti-6Al-4V/PAA/JI-2 specimens. It is important to mention, the consistency of the impedance data and compliance to the validation criteria for these measurements were assessed using KK transform. Figure 2 shows the EIS spectra obtained for Ti-6Al-4V alloy at different immersion times. These results are presented by Bode (Fig. 2 (a-b)) and Nyquist (Fig. 2c) diagrams. Thus, it is possible to notice that there were no significant spectral changes regardless of the immersion time. In the intermediary frequency regions of the spectrum, it can be seen that the phase angle remained close to -90\(^\circ\). The literature indicates that this behaviour is related to the good capacitive response of the metallic alloy [30–33]. Assis and Costa [32] evaluated the electrochemical properties of different titanium alloys (Ti-13Nb-13Zr, Ti-6Al-7Nb and Ti-6Al-4V) after long periods of immersion in Hank’s solution. In 410 days of immersion, no significant changes were observed in the EIS spectra for any of the studied alloys, showing the high corrosion resistance of these materials in Hank’s solution. These authors' findings corroborate the results found here, in which there were no changes in the EIS spectra after long periods of immersion. In fact, these findings are already well known for the Ti-6Al-4V alloy, since the corrosion products end up sealing electroactive areas and increasing the impedance modulus values of the mentioned alloy.

To describe the corrosion behaviour of the Ti-6Al-4V alloy, the equivalent electrical circuit (EEC) depicted in Fig. 2 (d) was used. The EEC adopted for the Ti-6Al-4V samples is also used by other works in the literature that seek to understand the electrochemical properties of this material in different electrolytes [34–35]. The components of this equivalent circuit are solution resistance (\(R_o\)), the polarization resistance of the barrier layer (\(R_p\)) as well as a constant phase element accounting for the capacitance of the barrier layer (CPE\(_{bl}\)).

The behaviour of this material is not fully capacitive, so it was necessary to use a constant phase element (CPE) in the equivalent circuit [30]. Thus, the impedance of the CPE element can be defined by Eq. 1 below:

\[
\frac{1}{\varvec{Y}_0 (\varvec{X}_0^{-\varvec{f}})} = \frac{1}{\varvec{Y}_0 (\varvec{X}_0^{-\varvec{f}}) \varvec{X}_0^{-\varvec{f}}} = \frac{1}{\varvec{Y}_0 (\varvec{X}_0^{-\varvec{f}}) \varvec{X}_0^{-\varvec{f}}}
\]
where \( Y_0 \) is the admittance and \(-1 \leq n \leq 1\), the \( n \) values being directly linked to the non-uniform current distribution on the material’s surface, which may be assigned to the presence of defects as well as roughness.

Table 1 shows the results obtained from the adjustments made for the non-functionalized Ti-6Al-4V alloy. In this sense, all adjustments performed showed a good correlation with \( \chi^2 \) values in the order of \( 10^{-4} \) [30]. As can be seen in Fig. 3, the \( R_0 \) values remain practically constant and as reported by Moreto et al. [36], this effect may be directly related to the distance between the working electrode and the reference electrode which is kept constant during the electrochemical tests. With the increase of the immersion time, there is a reduction in CPE\( _{bl} \) values and, consequently, an increase in \( R_{bl} \) (Fig. 3 (b-c)). At first, more generally, with the increase of immersion time, the TiO\(_2\) thin film ends up sealing the electroactive areas and improving the resistance to the corrosion process of the Ti-6Al-4V alloy. On the other hand, considering that more important than the exact CPE\( _{bl} \) values are the mechanistic information, it can be inferred that the CPE\( _{bl} \) values can be seen as capacitances. Thus, the decrease in capacitance values with the immersion time is related to the sealing of electroactive areas and the increase of the TiO\(_2\) thickness on the material’s surface.

![Image](image-url)

**Table 1**

<table>
<thead>
<tr>
<th>Material</th>
<th>Immersion time</th>
<th>( R_0 ) (( \Omega ) cm(^2))</th>
<th>CPE( _{bl} ) (( \mu F ) cm(^{-2}) S(^{-1}))</th>
<th>( n )</th>
<th>( R_{bl} ) (( \Omega ) cm(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ti-6Al-4V</td>
<td>3 h</td>
<td>40.49</td>
<td>38.30</td>
<td>0.88</td>
<td>1.01 x 10(^6)</td>
</tr>
<tr>
<td></td>
<td>6 h</td>
<td>39.48</td>
<td>36.30</td>
<td>0.89</td>
<td>1.10 x 10(^6)</td>
</tr>
<tr>
<td></td>
<td>9 h</td>
<td>38.27</td>
<td>35.10</td>
<td>0.89</td>
<td>1.13 x 10(^6)</td>
</tr>
<tr>
<td></td>
<td>12 h</td>
<td>38.35</td>
<td>33.90</td>
<td>0.89</td>
<td>1.17 x 10(^6)</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>39.70</td>
<td>31.20</td>
<td>0.89</td>
<td>1.43 x 10(^6)</td>
</tr>
<tr>
<td></td>
<td>168 h</td>
<td>38.28</td>
<td>27.80</td>
<td>0.90</td>
<td>3.30 x 10(^6)</td>
</tr>
</tbody>
</table>

Figures 4 and 5 show the morphologies of the Ti-6Al-4V alloy before and after the EIS tests exposed to 168 h in 0.6 mol L\(^{-1}\) NaCl solution. The SEM images demonstrated no visible corrosion attacks as well as corrosion products on the Ti-6Al-4V alloy. These findings corroborate the EIS results presented in Fig. 4, showing the good resistance of the Ti-6Al-4V alloy to the corrosion process for all immersion times. EDX analysis of the Ti-6Al-4V alloy surfaces (see Figs. 5 (a, b)) suggests that no changes have occurred in the chemical composition of the Ti-6Al-4V surfaces after the EIS tests.

EIS results for the Ti-6Al-4V/PAA/JI-2 are shown in Fig. 6 (a-c). An overview of the impedance spectrum indicates two-time constants: one related to the inner layer at low frequencies, and the second one at high frequencies to the outer layer. As shown, the EIS moduli decrease with the immersion time, indicating a removal of the Ti-6Al-4V/ PAA/ JI-2 on the Ti-6Al-4V alloy surfaces. The functionalized specimen presented a phase angle near −60°, for the lowest frequencies range (10\(^{-2}\) – 10\(^{-1}\) Hz) during all the immersion time.

The EEC used for the Ti-6Al-4V/PAA/JI-2 adjustment is presented in Fig. 7. The EEC is composed by the solution resistance (\( R_0 \)), barrier layer resistance (\( R_{bl} \)), additional resistance of the solution inside the porous (\( R'_{p} \)), a constant phase element accounting for the capacitance of the barrier layer (CPE\( _{bl} \)) as well as a constant phase element of the porous layer (CPE\( _{pl} \)).

Table 2 presents the parameters obtained by adjusting the EIS spectra for the Ti-6Al-4V/PAA/JI-2 specimens. The \( n_{bl} \) values are between 0.73–0.85, while the \( n_{pl} \) values are between 0.75–0.80. The solution resistance values are very close (\( R_0 \approx 41.48 \pm 0.92 \Omega \) cm\(^2\)) and as discussed earlier this is to be expected. As can be seen in Table 2, the barrier layer resistance (\( R_{bl} \)) exhibits higher values when compared to the additional resistance of the solution inside the porous (\( R'_{p} \)), suggesting a better corrosion performance (see Fig. 8). Figure 9 shows the adjustment performed in 3 h of immersion for the coated and uncoated specimens, showing the fitting quality as well as the good choice of the EEC. Figure 10 shows the comparison of impedance moduli values as a function of immersion time for the studied materials. It is possible to notice that up to 5 h of immersion, the peptide-containing sample presents a higher impedance value in comparison to the base material. However, these values decrease reaching 3.26 x 10\(^{-5}\) \( \Omega \) cm\(^2\) in 168 h of immersion. This observation may be related to the removal of PAA/JI-2 coating from the metallic material surfaces. In contrast, the base material displayed an inverse behaviour, and probably, this finding is due to the formation of TiO\(_2\) thin film on the metallic surface, reaching a value around 6.77 x 10\(^{-5}\) \( \Omega \) cm\(^2\) in 168 h of immersion.
The global corrosion resistance of the Ti-6Al-4V-1Zr alloy in comparison with Ti-6Al-4V alloy in Ringer-Brown solution and artificial Carter-Brugirard saliva of different pH values was studied by Poppa et al. [39]. The results presented by the authors only confirm those obtained in this present work, demonstrating the $R_p$ parameter presents a better resistance to the corrosion process in medium containing chloride ions when compared to the $R_p'$. In line with our results, we can cite the work developed by Toptan and collaborators [40]. The mentioned authors studied the corrosion behaviour of the SLM-produced Ti-6Al-4V alloy in comparison with its hot pressed and commercial counterparts by using EIS technique and demonstrated the $R_{bi}$ was noticeable higher when compared to the $R_p$ for all specimens, suggesting once again the best resistance to the corrosion process.

The CPE$_{bi}$ values increase with the immersion time, suggesting a decreasing on the corrosion performance of the porous layer. In fact, this hypothesis may be supported by the SEM images obtained after the EIS tests and displayed in Figs. 11 (a-c). The proposed corrosion mechanism for the functionalized samples is described in Fig. 11 (q). When the Ti-6Al-4V/PAA/JI-2 specimens are exposed to the aggressive medium (step I) the aggressive ions penetrate through the imperfections of the coating (step II). As the immersion time increases, the coating deposited on the surface Ti-6Al-4V alloy surface begins to be removed (step III), forming these ring-shaped irregularities on the surface of the functionalized material. At this moment, the coating already has an irregular surface when compared to the initial test times. At this stage, there is a surface composed of covered and uncovered regions (step IV), corroborating the image shown in Fig. 11 (b). Therefore, up to 168 h of immersion, the corrosion resistance of the functionalized Ti-6Al-4V alloy may be attributed as a synergistic process due to the coated region with PAA/JI-2 and the formation of TiO$_2$ films on the uncovered regions. With respect to the CPE$_{bi}$ parameter, it can be seen an increase in their values with the immersion times, which may be related due to i) a general attack to the oxide film, ii) a decrease in the protective layer with the immersion time or due to the iii) hydration of the oxide film upon immersion. In fact, these observations are clarified when the CPE's values are transformed into capacitance as will be discussed in the next paragraphs.

The CPE$_{pl}$ values were converted to the capacitance of the native oxide film (C$_{ox}$) by using the mathematical relationship, derived from Brug's equation (Equations 2) [42]. Thereby, combining the values obtained by the Eq. (2) and substituting them in Eq. (3), it is possible to determine the thickness of the native oxide layer (Eq. 4).

### Table 2

<table>
<thead>
<tr>
<th>Material</th>
<th>Immersion time</th>
<th>$R_O$ (Ω cm$^2$)</th>
<th>CPE$_{bi}$ (µF cm$^{-2}$s$^{0.5-1}$)</th>
<th>$n_{bi}$</th>
<th>CPE$_{pl}$ (µF cm$^{-2}$s$^{0.5-1}$)</th>
<th>$n_{pl}$</th>
<th>$R'_P$ (Ω cm$^2$)</th>
<th>$R_{bi}$ (Ω cm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ti-6Al-4V/PAA/JI-2</td>
<td>3 h</td>
<td>41.32</td>
<td>48.30</td>
<td>0.85</td>
<td>8.79</td>
<td>0.80</td>
<td>67.44</td>
<td>6.35x10$^5$</td>
</tr>
<tr>
<td></td>
<td>6 h</td>
<td>40.77</td>
<td>59.00</td>
<td>0.84</td>
<td>10.50</td>
<td>0.78</td>
<td>94.55</td>
<td>6.95x10$^5$</td>
</tr>
<tr>
<td></td>
<td>9 h</td>
<td>40.43</td>
<td>59.60</td>
<td>0.84</td>
<td>11.70</td>
<td>0.78</td>
<td>104.90</td>
<td>7.07x10$^5$</td>
</tr>
<tr>
<td></td>
<td>12 h</td>
<td>40.63</td>
<td>80.40</td>
<td>0.81</td>
<td>11.00</td>
<td>0.78</td>
<td>169.50</td>
<td>8.09x10$^5$</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>43.34</td>
<td>81.20</td>
<td>0.80</td>
<td>11.33</td>
<td>0.79</td>
<td>300.30</td>
<td>8.49x10$^5$</td>
</tr>
<tr>
<td></td>
<td>168 h</td>
<td>42.38</td>
<td>147.00</td>
<td>0.73</td>
<td>11.34</td>
<td>0.77</td>
<td>1033.00</td>
<td>9.60x10$^5$</td>
</tr>
</tbody>
</table>

Figures 12 (a) presents the capacitance values obtained from the Brug's equation in the present work, as well as the behaviour of the barrier layer as function of the immersion times for the Ti-6Al-4V/PAA/JI-2. As shown, the capacitance values increase with the immersion time, suggesting a decreasing in the thickness of the native oxide layer. As reported by the literature [43–45], the dielectric constant of the TiO$_2$ formed on the titanium and its alloys surfaces exhibit values between 48 and 100, respectively. In this sense, considering the free space permittivity as $\varepsilon_0 = 8.854 \times 10^{-14}$ F cm$^{-1}$ and the dielectric constant ($\varepsilon$) is equal to 48, the thickness evolution of the native oxide layer as function of immersion time may be represented in Eq. 5. As can be seen, the thickness of the oxide layer at the beginning of the tests is approximately 2.22 nm, decreasing with immersion time ($\sim$ 0.52 nm for the 168 h of immersion). On the other hand, this value varies with the increase of the dielectric constant. Thus, considering intermediate values between 48 and 100, the oxide thicknesses are obtained according to the literature [7].
Recently, it was demonstrated by scanning vibrating electrode technique (SVET) associated to the morphological analyses that the localised behaviour of the Ti-6Al-4V/PAA/JI-2 specimens could be directly related to the presence of peptide agglomerates, which can intensify the corrosion process due to their charge properties [19]. The results obtained through the electrochemical impedance spectroscopy technique confirm the previously verified observations reinforcing that the electrostatic attraction between the ionic species and the peptide rich-agglomerates might be the responsible for intensifying the corrosion behaviour of the biomaterial. Trino et al. [23] showed that surfaces in which the layer of peptide immobilized are uniformly distributed result in better corrosion properties than those in which clusters of peptides are observed. As already presented by this research group in reference [46], several factors can contribute to the formation of PAA thin films on the Ti-6Al-4V alloy surfaces, such as: power, frequency, pressure, temperature, voltage, accuracy of the equipment used, among others. Thus, the surface homogeneity results from a totally synergistic process that directly affects the distribution and uniformity of the PAA and, consequently, the immobilization of the peptides. Figure 13 exhibits an overview of the Ti-6Al-4V alloy functionalized with PAA/JI-2 after the electrochemical impedance tests (168 h of immersion in sodium chloride solution). One can verify the existence of peptides dispersion along the metallic matrix (see Fig. 13 (a)). Although the samples were metallized with Au nanoparticles, seeking better electronic conduction on the material's surface, the images obtained still do not have the same quality as the base material. A magnification of the previous image is presented in Fig. 13 (b), showing a peptide of approximately 0.8 µm in size. An increase in carbon concentration at distances between 2.8 and 3.2 µm (see maps in Fig. 13 (c)), confirms the peptide agglomerates formation. Although the functionalization process by using PECVD technique was successful in immobilizing the antimicrobial peptide JI-2 in the Ti-6Al-4V alloy and appears as a simple method of surface treatment, it becomes necessary to develop further studies, so that the peptides can be uniformly distributed over the Ti-6Al-4V alloy matrix and positively influence the corrosive properties of the functionalized material.

4. Conclusions

In the present work, the global electrochemical behaviour of the Ti-6Al-4V alloy functionalized with AMP JI-2 was studied at different immersion times in 0.6 mol L\(^{-1}\) NaCl solution. Here, the PECVD technique was used to form thin films of PAA containing carboxyl groups for subsequent peptide immobilization with great prospects on the biomedical sector. The PECVD technique was advantageous for the functionalization of the Ti-6Al-4V alloy widely used as biomaterial. Results demonstrated that Ti-6Al-4V/PAA/JI-2 thin films shifted the corrosion potential (E\(_\text{corr}\)) to more positive values, initially indicating a better thermodynamic stability than the base material. These samples also displayed higher impedance moduli when compared to the base material in the first hours of immersion. However, as the immersion time increases, PAA/JI-2 coating begins to be removed from the metallic surface. The capacitance of the barrier layer (C\(_\text{bl}\)) increased for the coated specimens with increasing immersion time, suggesting a decreasing in the thickness of the native oxide layer. The opposite effect was observed for the capacitance of the porous layer, demonstrating that peptides distribution influence on the materials' electrochemical properties. The EEC used for fitting the EIS experimental data allowed to obtain a thickness for the native oxide of approximately 2.22 nm (ε = 48) and 4.63 (ε = 100) at the beginning of the electrochemical tests. Finally, these results indicate that despite the advantages of the chosen surface treatment it intensified the corrosion process on the material. Finally, this work presents the versatility of surface treatments by allowing to modulate several applications. By indicating that an improvement in the used methodology for peptide immobilization should be made, to avoid peptide agglomerates and the corrosive behaviour associated with them. Nevertheless, considering the biological properties, charge agglomerates are interesting due to the anionic character of bacterial cell membranes, a premise that underlies the action of antimicrobial peptides.

Declarations

Author Statement

The authors' contributions are:

Teixeira, G. T. L. – Investigation;

Gelamo, R. V. – Investigation and Formal analysis;

Leite, N. B. – Project administration, Supervision, Investigation, Formal analysis, and writing-up;

Moreto, J. A. – Project administration, Supervision, Investigation, Formal analysis, and writing-up;

Conflict of interest

All authors declare that they have no conflict of interest.

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Data availability
The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability
Not applicable

References


Figure 1

OCP measurements in 0.6 mol L\textsuperscript{-1} NaCl for Ti-6Al-4V and Ti-6Al-4V/PAA/ JI-2 specimens.
Figure 2

EIS measurements in 0.6 mol L\(^{-1}\) NaCl for the Ti-6Al-4V alloy, (a-b) Bode diagram, (c) Nyquist diagram, and (d) Proposed EEC.
Figure 3

Values of the EIS parameters of the Ti-6Al-4V alloy, (a) $R_{\Omega}$, (b) CPE, (c) $R_{sl}$ as a function of immersion time.
Figure 4

Morphological characteristics of the uncoated surfaces (a-c) before the EIS assays, and (d-g) after the EIS assays. Images were obtained by secondary electron (SE) at magnifications of 1000 and 5000x.
Figure 5

Analysis of the chemical composition of the uncoated surface (a) before the EIS tests, and (b) after the EIS tests.
Figure 6

EIS measurements in 0.6 mol L$^{-1}$ NaCl solution for the Ti-6Al-4V/PAA/JI-2. (a-b) Bode diagram and (b) Nyquist diagram.

Figure 7

(a) Schematic representation of the corrosion and the EEC proposed by Jüttner [37], and (b) EEC used for fitting the experimental data proposed by Mansfeld and Kendig [38].
Figure 8

Values of the EIS parameters of the Ti-6Al-4V/PAA/JI-2 (a) $R_0$ (b) CPE$_{bl}$ (c) CPE$_{pl}$ (d) $R'_p$ (e) $R_{bi}$ as a function of immersion time.

Figure 9

Adjustments obtained for the 3 h of immersion time in 0.6 mol L$^{-1}$ NaCl solution.
Figure 10
Impedance moduli behaviour for the uncoated and coated specimens obtained at 0.01 Hz as a function of immersion time.

Figure 11
Morphological characteristics of coated surfaces, (a) before EIS tests, (b) after EIS tests, (c) corrosion mechanism proposed for functionalized samples. Images were obtained by secondary electron (SE) at magnification of 20000x.
Figure 12

(a) $C_{bl}$ behaviour as function of immersion time for the and Ti-6Al-4V/PAA/JI-2 specimens and (b) the thickness evolution of the native oxide layer as function of immersion time.

Figure 13
(a) An overview of the Ti-6Al-4V alloy functionalized with PAA/JI-2 after the EIS tests (168 h of immersion in sodium chloride solution), (b) peptide presence on the functionalized Ti-6Al-4V surface and, (c) chemical composition obtained from EDX analysis, showing the presence of Ti, Al, V and C elements.

**Supplementary Files**

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