The Synergistic Effect of Proteins and Reactive Oxygen Species on Electrochemical Behaviour of 316L Stainless Steel for Biomedical Applications

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Abstract. The stainless steels, especially 316L type is the most used metallic biomaterials for biomedical applications due to their good biocompatibility, low price, excellent corrosion resistance, availability, easy processing and high strength. Due to these favorable properties 316L stainless steel has become the most attractive biomaterial for dental implants, stents and orthopedic implants. However an implant material in the human body is exposed to an action effect of other molecules, including proteins (such as albumin) and reactive oxygen species (such as hydrogen peroxide - $H_2O_2$) produced by bacteria and immune cells. In the literature there are few studies to follow the effect of proteins and reactive oxygen species on 316L stainless steel used as implant material and are still unclear. The degree of corrosion resistance is the first criterion in the use of a metallic biomaterial in the oral or body environment. The aim of this research work is to investigate the influence of proteins (albumin) and reactive oxygen species ($H_2O_2$) in combination, taking into account the synergistic effect of these two factors on 316L stainless steel. Albumin is present in the body near implants and reactive oxygen species could appear in inflammatory processes as well. The study shows that the presence of albumin and reactive species influences the corrosion resistance of 316L stainless steel in biological solutions. In this research work the corrosion behavior of 316L stainless steel is analyzed by electrochemical methods such as: open circuit potential (OCP), Electrochemical Impedance Spectroscopy (EIS). It was found that, the electrochemical results are in a good agreement with micro photographs taken before and after corrosion assays. The albumin and reactive oxygen species have influence on 316L stainless steel behavior.

1. Introduction
Metallic biomaterials used in biomedical applications include stainless steel, especially 316L type, which is the most used metallic biomaterial for biomedical applications due to their good biocompatibility, low price, excellent corrosion resistance, availability, easy processing and high strength.

Due to these favorable properties 316L stainless steel has become the most attractive biomaterial for dental implants, stents and orthopedic implants. The primary limitation of this metallic biomaterial is the release of toxic metal ion, which can lead to various side tissue reactions [1].

Understanding the chemical and biological requirements for metallic biomaterials is an essential condition, so it is necessary to discuss possible reactions between corrosion products and the human body. In the event of corrosion products occurring on the metallic implant introduced into the human body, the formation of a new compound that is not found in the materials used in the implant,

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body, the flux of electrons in the metal leads to an ion flux around the tissue, resulting in a nerve cell disruption, which is undesirable because the metal ions released from the cause of inorganic corrosion reactions is transported to internal organs such as the liver or kidney.

In the continuous corrosion process, these ions accumulate in the internal organs, leading to other possible diseases, if the toxicity limit is exceeded. An organic reaction, for example a metal reaction with proteins, can lead to allergic or inflammatory reactions of the surrounding tissue. Albumin is the most common protein in the peri-implant environment and ROS (reactive oxygen species) such as \( H_2O_2 \) are produced in the presence of inflammation reaction, which are common after surgery, or in response to infection or corrosion products [2].

In the literature there are few studies to follow the effect of proteins and reactive oxygen species on 316L stainless steel used as implant material and are still unclear [3]. The purpose of this study was to evaluate the synergistic effect of proteins and reactive oxygen species on electrochemical behavior of 316L stainless steel exposed in Phosphate Buffered Saline solution (PBS) pH 7.32 with addition of albumin (1 mL/L), \( H_2O_2 \) (1 mL/L) and proteins (albumin) and reactive oxygen species (\( H_2O_2 \)) in combination in view of biomedical applications.

2. Experimental procedures
2.1. Materials and methods
The corrosion experiments were performed on 316L stainless steel medical grade. The chemical composition of 316L stainless steel is presented in Table 1.

Table 1. The chemical composition of 316L stainless steel (wt %).

| Element / wt% | C     | Cr   | Ni   | Mo   | Si   | Mn   | Fe   |
|---------------|-------|------|------|------|------|------|------|
| 316L          | 0.03  | 16.5-18.5 | 11-14 | 2.0-2.5 | 1.0  | 2.0  | Balance |

Table 2. The chemical composition of biological solutions.

| Nr. Crt. | Compound | Phosphate Buffered Saline (PBS) g / L | Phosphate Buffered Saline (PBS) g / L | Phosphate Buffered Saline (PBS) g / L | Phosphate Buffered Saline (PBS) g / L |
|----------|----------|--------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|
| 1.       | NaCl     | 8.0                                  | 8.0                                  | 8.0                                  | 8.0                                  |
| 2.       | KCl      | 0.2                                  | 0.2                                  | 0.2                                  | 0.2                                  |
| 3.       | Na\(_2\)HPO\(_4\) | 1.14                           | 1.14                           | 1.14                             | 1.14                             |
| 4.       | KH\(_2\)PO\(_4\) | 0.27                           | 0.27                           | 0.27                             | 0.27                             |
| 5.       | 30% \(H_2O_2\) | -                                 | 1 mL / L                         | -                                 | 1 mL / L                         |
| 6.       | Albumin  | -                                    | Balance                           | Balance                           | Balance                           |
| 7.       | Deionized water | Balance                       | Balance                           | Balance                           | Balance                           |
| 8.       | pH       | 7.32                                 | 7.32                               | 7.32                               | 7.32                               |

In order to assess the corrosion behavior of 316L stainless steel a conventional three-electrode cell was used, consisting of: the working electrode (WE) made of 316L stainless steel, an Ag/AgCl reference electrode with KCl saturated solution (E = +199 mV vs. standard hydrogen electrode – SHE) and a Pt-Rh grid as auxiliary electrode. Before corrosion experiments, the squares specimens for electrochemical measurements were connected to a copper wire for electrical connection and insulated with epoxy resin leaving a working area of 2.25 cm\(^2\). The working surface was cleaned with alcohol and acetone and rinsed with distilled water.
The corrosion experiments were done using a Potentiostat–Galvanostat PGZ 100 and the experimental data were recorded with VoltaMaster4 software. The electrochemical measurements such as Open Circuit Potential (OCP), and Electrochemical Impedance Spectroscopy (EIS) were carried out to access the anticorrosive characteristics of the 316L stainless steel medical grade.

Corrosion tests were performed for 316L stainless steel in Phosphate Buffered Saline (PBS) solution [4]. The chemical composition of biological solution it is shown in Table 2.

In Table 3 are shown the physico-chemical characteristics of biological solution which was measured with the multi-parameter analyzer CONSORT C 533.

### Table 3. Biological solution characteristics.

| Biological solution         | pH   | Salinity [%] | Conductivity [mS] |
|-----------------------------|------|--------------|-------------------|
| Phosphate Buffered Saline   | 7.32 | 8.20         | 14.5              |

#### 3. Results and discussions

**3.1. Open circuit potential (OCP)**

The open circuit potential measurements indicate the tendency of a material to electrochemical oxidation in a corrosive environment. The potential time measurements of 316L stainless steel medical grade immersed in Phosphate Buffered Saline solution (PBS) are shown in (Figure 1).

![Figure 1. Open circuit potential plots of the 316L stainless steel immersed in: (1) Phosphate Buffer Saline, (2) PBS + 1 mL/L H$_2$O$_2$, (3) PBS + 1 mL/L albumin and (4) PBS + 1 mL/L H$_2$O$_2$ + 1 mL/L albumin during 60 minutes of immersion time.](image)

From (Figure 1) it can be seen that 316L stainless steel immersed in PBS solution, showed a slow shifted value of open circuit potential in the more negative (active) direction, having at the end of 60 min. the value +23.19 mV vs. Ag/AgCl, compared with the initial immersion time value of +239.88 mV vs. Ag/AgCl. For stainless steel immersed in PBS solution with addition of 1 mL/L albumin, the open circuit potential shows a decreasing value from +288.25 mV vs. Ag / AgCl at initial immersion time to +53.07 mV vs. Ag / AgCl at the end of measurement.
The shift of the potential to a negative direction indicates that the material is not able to form a protective oxide layer on its surface after the exposure under the biological solution. For stainless steel immersed in PBS solution with addition of 1 mL/L $H_2O_2$, the open circuit potential show the same shifted trend to more active values from +294.38 mV vs. Ag/AgCl measured at initial immersion time to +256.57 mV vs. Ag/AgCl at the end of monitoring time.

The OCP values of samples immersed in PBS solution with addition of albumin (1 mL/L) and $H_2O_2$ (1 mL/L) in combination, showed a very slow shifted value in the more negative (active) direction, having at the end of 60 min. the value +259 mV vs. Ag/AgCl, compared with the initial immersion time value of +298.13 mV vs. Ag/AgCl.

Also it can be seen from (Figure 1) that the open circuit potential steady state is attained for 316L stainless steel immersed in PBS solution with addition of mixed $H_2O_2$ (1 mL/L ) and proteins (albumin) (1 mL/L ) after 5 minutes of immersion into electrolyte.

3.2. Electrochemical impedance spectroscopy

The electrochemical impedance spectroscopy measurement (EIS) is a non-destructive technique and was performed to characterize the corrosion behavior of 316L stainless steel immersed in PBS solution, PBS solution with addition of albumin (1 mL/L), $H_2O_2$ (1 mL/L) and albumin and reactive oxygen species ($H_2O_2$) in combination.

The EIS spectra were recorded in the frequency range from 100 kHz to 0.2 Hz, with amplitude of the sine-wave signal of 10 mV. The impedance data recorded for 316L stainless steel immersed in all four solutions were displayed in the form of a Nyquist plot in (Figure 2).

![Figure 2](image_url)

**Figure 2.** The Nyquist representation of EIS specters recorded for SS316L immersed in (1) Phosphate Buffer Saline, (2) PBS + 1 mL/L $H_2O_2$, (3) PBS + 1 mL/L albumin and (4) PBS + 1 mL/L $H_2O_2$ + 1 mL/L albumin. The plain symbols represent the experimental data and the continuous lines represent the fitted results.

The EIS spectra were fitted using ZView 3.4f software and the quality of the fitted results was evaluated with the chi-square value that was lower than $10^{-3}$. 

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The EIS spectra recorded for 316L stainless steel immersed in PBS electrolyte can be characterized by a large semicircle capacitive loop. The EIS specters recorded for 316L stainless steel tested in PBS with 1 mL/L H₂O₂, PBS with 1 mL/L albumin and PBS with 1 mL/L H₂O₂ and 1 mL/L albumin present a small capacitive semicircle at high frequency and a large semicircle capacitive loop at low frequency.

For 316L stainless steel immersed in PBS solution, the EIS spectra was fitted using a simple Randles equivalent circuit (showed in Figure 3 (a)) composed by Rs is the electrolyte resistance, CPEr and Rr are the constant phase element and the resistance of 316L stainless steel base metal.

In order to fit the EIS specters recorded for 316L stainless steel immersed in PBS with 1 mL/L H₂O₂, PBS with 1 mL/L albumin and PBS with 1 mL/L H₂O₂ and 1 mL/L albumin it was used a complex equivalent circuit presented in (Figure 3 (b)) where Rs was designated the electrolyte resistance, CPEo and Ro are the constant phase element and polarization resistance of oxide layer formed in the presence of H₂O₂ or the albumin layer adsorbed on 316L stainless steel surface during the oxidation of [5], CPEb and Rb are the constant phase element the resistance of the base metal.

The constant phase element was used instead of pure capacitor because the non-homogeneity of analyzed samples and non-uniform current distribution caused by the surfaces roughness [6].

Accordingly with A. Lasia [7], the impedance of constant phase element is defined as:

$$Z_{CPE} = \frac{1}{Q(j\omega)^\alpha}$$

where: $j = \sqrt{-1}$ is an imaginary number, $\omega$ represent the angular frequency, $Q$ is a frequency independent parameter and $\alpha$ is an exponential factor ranged between -1 and 1.

![Figure 3. Schematic representation of equivalent electric circuits of 316L stainless steel surfaces for fitting and simulation of electrochemical impedance spectroscopy experimental data: (a) Randles equivalent circuit and (b) complex equivalent circuit.](image)

The specific polarization resistance determinated using the proposed equivalent circuits reveal that for 316L stainless steel immersed in PBS solution, the specific polarization resistance value is around 19930 kohm cm². The addition of 1 mL/L H₂O₂ into PBS electrolyte increase the rate of anodic and cathodic reactions and the specific polarization resistance decrease up to 1222 kohm cm². On the other hand, the addition of 1 mL/L albumin in PBS electrolyte cause the inhibition of anodic and cathodic reactions and the specific polarization resistance increase up to 24970 kohm cm². In the presence of both, H₂O₂ and albumin in PBS electrolyte, the specific polarization resistance of 316L stainless steel decrease up to 1431 kohm cm² in comparison with the specific polarization resistance of 316L stainless steel immersed in PBS electrolyte. The H₂O₂ and albumin added into PBS electrolyte increase the rate of anodic reaction simultaneously with the inhibition of cathodic reaction rate and increase the corrosion rate of 316L stainless steel.
Similar results were obtained by F. Yu and his co-workers [3] when evaluated the synergetic effect of bovine serum albumin and H$_2$O$_2$ added in physiological solution on corrosion behavior of Ti6Al4V.

3.3. Optical Microscopy analysis before and after corrosion
The surfaces of 316L stainless steel were investigated before and after corrosion tests in Phosphate Buffer Saline, PBS + 1 mL/L H$_2$O$_2$, PBS + 1 mL/L albumin and PBS + 1 mL/L H$_2$O$_2$ + 1 mL/L albumin in order to estimate the corrosive effects and are presented in (Figure 4).

Figure 4. Optical microscopy of stainless steel 316L (a) before corrosion, (b) after corrosion in Phosphate Buffer Saline, (c) after corrosion in PBS + 1 mL/L H$_2$O$_2$, (d) after corrosion in PBS + 1 mL/L albumin and (e) after corrosion in PBS + 1 mL/L H$_2$O$_2$ + 1 mL/L albumin.
Figure 4 (a) shows that before corrosion tests the 316L stainless steel has a uniform surface with no defects. From Figure 4 (b) after corrosion in Phosphate Buffered Saline and 4 (d) after corrosion in PBS + 1 mL/L albumin 316L stainless steel surface are the least affected by pitting corrosion.

The samples of 316L stainless steel immersed in PBS + 1 mL/L H$_2$O$_2$ + 1 mL/L albumin (Figure 4 (e)) suffer a corrosion at the surface of the material while the 316L stainless steel surface immersed in PBS + 1 mL/L H$_2$O$_2$ present pitting damage (Figure 4 (c)), the pitting damage covers a high surface of the 316L stainless steel sample and the pits are deeper into the substrate. The optical microscopy images are in good agreements with electrochemical measurements.

4. Conclusions

The aim of this research paper is to investigate the influence of proteins (albumin) and reactive oxygen species (H$_2$O$_2$) and as well as their influence in combination, taking into account the synergistic effect of these two factors on 316L stainless steel in Phosphate buffered saline solution.

The experimental results reveal that the addition of 1 mL/L H$_2$O$_2$ into PBS electrolyte increase the rate of anodic and cathodic reactions and the specific polarization resistance decrease up to 1222 kohm cm$^2$. On the other hand, the addition of 1 mL/L albumin in PBS electrolyte cause the inhibition of anodic and cathodic reactions and the specific polarization resistance increase until 24970 kohm cm$^2$. In the presence of both, H$_2$O$_2$ and albumin in PBS electrolyte, the specific polarization resistance of 316L stainless steel decrease up to 1431 kohm cm$^2$ in comparison with the specific polarization resistance of 316L stainless steel immersed in PBS electrolyte.

The H$_2$O$_2$ and albumin added into PBS electrolyte increase the rate of anodic reaction simultaneously with the inhibition of cathodic reaction rate and increase the corrosion rate of 316L stainless steel.

From the optical microscopy analysis of 316L stainless steel surfaces after corrosion immersed in Phosphate Buffered Saline solution and PBS + 1 mL/L albumin are the least affected by pitting corrosion. The samples of 316L stainless steel immersed in PBS + 1 mL/L H$_2$O$_2$ + 1 mL/L albumin suffer a corrosion at the surface of the material while the 316L stainless steel surface immersed in PBS + 1 mL/L H$_2$O$_2$ present pitting damage and the pits are deeper into the substrate.

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