The Effect of H$_2$O$_2$ and Lactic Acid Addition in Biological Saliva on the Corrosion Behaviour of 304L Stainless Steel

N Simionescu$^1$, L Benea$^{1*}$ and A Chiriac$^{1,2}$

$^1$ Competences Center: Interfaces-Tribocorrosion-Electrochemical Systems (CC-ITES)
Faculty of Engineering, Dunarea de Jos University of Galati, 47 Domneasca street, Galati, Romania
$^2$ Faculty of Medicine and Pharmacy, Dunarea de Jos University of Galati, 35 Alexandru Ioan Cuza Street, RO-800010 Galati, Romania

*E-mail: lidia.benea@ugal.ro

Abstract. The aim of this study is to assess the corrosion resistance of SS304L in biological solution (named Ringer) at body temperature 37$^\circ$C. The addition of H$_2$O$_2$ and C$_3$H$_6$O$_3$ simulate the presence of microorganism metabolic product and inflammatory conditions. 304L stainless steel, in the oral cavity, can interact with other product capable of initiating corrosion of metals. H$_2$O$_2$ was used because is delivered by leukocytes and microscopic organism (bacteria) occurs in conditions in which the human body suffers a process of inflammation and lactic acid can be produced by a bacterial strain called Streptococcus mitis meeting in the dental plaque. For corrosion investigations was used in situ measurement (electrochemical) such as: OCP (open circuit potential) and EIS (electrochemical impedance spectroscopy). The electrochemical results conclude that the additions of hydrogen peroxide and lactic acid as well as these two compounds in combination have influence on the corrosion behaviour of SS304L in Ringer saliva.

1. Introduction
SS304L is progressively used within the field of biomaterials. The biocompatibility reported in various researches has led to the use of 304 type L steels in medical applications [1], especially in orthopedics for fixing fractures in the form of bone plates, intermediate nails and screws, fixed orthodontic appliances, dental crowns, tube of tracheostomy, among other non-implant medical devices and dental appliances [2, 3].

The way in which the human body responds to the phenomenon called corrosion when certain implant materials are placed in human body is one of the interesting areas [4].

The most common biomaterials implanted in the human body are derived from three systems of materials, namely: titanium alloys, cobalt chromium alloys and stainless steel [5]. After implantation of biomaterials in the human body, many problems can arise; one of them is the interaction between the material and the physiological material that can lead to implant failure.

Therefore it is important that the selected material implanted in the human body to be tested and explored in vitro in all possible situations because the human body is not a hospitable environment with metallic materials, this being a strongly oxygenated environment, with an ionic composition and concentration proteins from the fluid in the human body that complicate understanding of biomaterials and corrosion by default [6].
So corrosion remains one of the main problems facing the metal biomaterials market. Temperature and pH are other important factors that affect the behavior of metal biomaterials when inserted into the human body. So it is important that when these materials are tested in the laboratory they are tested at the internal temperature of the human body respectively 37°C [7].

It is also important to simulate the inflammatory process, or the microorganisms, or the synergistic effect of both because they can affect the corrosion behavior due to effects such as pH modification [7].

In the literature we find few studies that have followed the synergistic effect of albumin and hydrogen peroxide on the corrosion resistance of SS316L in solution (phosphate buffered saline solution) [8], on the corrosion investigations of titanium alloy (grade 5) in FM (Fusayama Meyer) saliva [9], the effect of NaF, H₂O₂ and C₃H₆O₃ on the corrosion behaviour of titanium grade 4 [10], but we did not find studies to follow the synergistic effect of H₂O₂ and lactic acid on the corrosion resistance of SS304L in Ringer saliva tested at body temperature. The objective of this work is to investigate the addition of H₂O₂ and C₃H₆O₃ (lactic acid) in combination on the corrosion resistance of SS304L in, immersed in Ringer solution by electrochemical methods like: OCP and EIS, at body temperature, with addition of H₂O₂ (10 g/L), lactic acid (C₃H₆O₃) (10 g/L) and H₂O₂ (10 g/L) and lactic acid (10 g/L) in combination.

2. Experimental procedures

2.1. Materials and methods

SS304L was purchased from Direct Line Inox (Bucharest) in the form of sheet. The size of sheet was 1000 mm x 1000 mm x 1.2 mm, for corrosion experiment were cut at 25 mm x 25 mm x 1.2 mm, for electrical contact were bonded with copper electrical cable, insulated with resin to have an measurable active surface of 4.25 cm². The values that correspond to the SS304L passed in the quality certificate issued by the producing company are presented in Table 1.

| Element / wt% | C      | Cr       | Ni       | Si       | Mn      | Fe       |
|---------------|--------|----------|----------|----------|---------|----------|
| SS304L        | 0.030  | 10.5-17.5| 8-10.5   | 1.0      | 2.0     | Balance  |

For the evaluation of the corrosion resistance of SS304L stainless steel, was used an electrochemical cell composed of three electrodes (SS304L used as WE (working electrode), Platinum-Rh wire as auxiliary electrode and Silver / Silver Chloride with potassium chloride saturated solution as RE (reference electrode) connected at a equipment PGZ 301 piloted with the help of data recording software VoltaMaster 4. The tests were performed at the temperature of the human body 37°C ± 1°C.

The electrolyte volume of the electrochemical cell used for each experiment was 150 mL, and each experiment was triplicate to verify the data reproducibility. Prior to each experiment, 304L stainless steel samples were cleaned with NaOH (50 g / L), HCl (1:1), rinsed with distilled water dry with filter paper and placed immediately in the test solution.

Electrochemical experiments were performed in the Ringer biological solution whose chemical composition is given in Table 2 and the interest parameters of Ringer solution was measured with an equipment (analyzer multi parameter) Sension+. All chemical reagents used were by p.a, purchased from Lachner chemical Co (Slovakia).

The hydrogen peroxide used to prepare tested solution was purchased from Lachner chemical Co (Slovakia) and have a concentration of 30%. Lactic acid (85 %) was purchased from Sigma Aldrich.
Table 2. The chemical composition of Ringer solution.

| Nr. Crt. | Reagents used | Ringer g / L | Ringer g / L | Ringer g / L | Ringer g / L |
|----------|---------------|--------------|--------------|--------------|--------------|
| 1.       | NaCl          | 8.402        | 8.402        | 8.402        | 8.402        |
| 2.       | KCl           | 0.302        | 0.302        | 0.302        | 0.302        |
| 3.       | CaCl₂         | 0.298        | 0.298        | 0.298        | 0.298        |
| 4.       | H₂O₂ (30 %)   | -            | -            | 10           | 10           |
| 5.       | Lactic acid (LA) (85 %) | -        | 10           | -            | 10           |
| 6.       | Distilled water | Balance     | Balance      | Balance      | Balance      |
| 7.       | pH            | 6.67         | 1.96         | 5.26         | 1.85         |
| 8.       | Conductivity [mS / cm] | 14.1     | 16.0         | 13.8         | 15.4         |
| 9.       | Salinity [ppt] | 8.1          | 9.2          | 8            | 8.9          |

3. Results and discussions

3.1. Open circuit potential (OCP)

The potential variation as a function in time in OCP measurement was followed for 180 minutes, with step measurement duration of 2 sec, until the equilibrium state of the potential for the SS304L tested in those four solutions is attained. This process was made to see the oxidation tendency of SS304L stainless steel in presence of Ringer solution, Ringer + 10 g/L lactic acid, Ringer + 10 g/L H₂O₂ and this two in combination.

![Open circuit potential graph](image)

In Figure 1 can be observed, that the variation of the OCP for the SS304L tested in the biological saliva solution at temperatures which simulating the temperature of the human body, starting from a potential value of -196.7 mV vs. Reference Electrode from immersion, having a slight increase and after about 60 minutes reaching at the end of the measurement at 155.43 mV vs. Ag/AgCl.

When 10 g/L lactic acid (LA) is added to the Ringer solution, it is observed that the value of the open circuit potential decreases slightly compared to the Ringer solution, starting from a potential of -215.51 mV vs. reference electrode having a slight increase, reaching at the final of 180 min. around of potential value -204.56 mV vs. reference electrode. This behavior reveals that the amount of lactic acid added in Ringer solution affects the dissolution of SS304L.
With 10 g/L H$_2$O$_2$ added in solution it is observed that there is an increase in the potential compared to the Ringer solution and the solution containing 10 g/L lactic acid, starting from a value of 40.32 mV vs. Reference electrode at initial time, having a tendency to shift the potential towards more negative (but positive values), reaching at the ending of 180 min. of monitoring the potential at 15.28 mV vs. Ag/AgCl. This behavior is possible due to the oxidizing effect of hydrogen peroxide, thus favoring the growth of the oxide film on the surface of 304L stainless steel. At the same time leading to the attack of the oxide layer formed by exposure to oxygen. This behavior was also supported by the EIS results which showed lower R$_p$ values for the SS304L stainless steel tested in Ringer solution with hydrogen peroxide added.

In the case of the Ringer solution with 10 g/L lactic acid + 10 g/L H$_2$O$_2$, there is an increase in the potential compared to the Ringer solution and compared with Ringer solution with lactic acid, but there is a decrease in the potential compared to the solution with 10 g/L H$_2$O$_2$. The open circuit potential starts from a potential of 47.71 mV vs. Reference electrode from immersion and reaches at the final of the measurement the potential value of -26.79 mV vs. Reference electrode. This behavior is also dependent on the fact that in the presence of lactic acid and H$_2$O$_2$ there was a decrease in pH. The results suggest the harmful effect that the synergistic effect of lactic acid and the H$_2$O$_2$ on the 304L stainless steel.

This behavior is sustained also by the lowest values obtained from the polarization resistance (EIS measurement) and microphotographs taken before and after corrosion test.

3.2. Electrochemical Impedance Spectroscopy (EIS)

EIS is powerful and precise methods which gain interest in recent decades, successfully applied to the study of corrosion systems. The EIS spectra it was made at free potential in a range of frequency started from the 100 kHz to 10 mHz, with a signal perturbation of amplitude of 10 mV. The EIS spectrum was fitted using software ZView 3.4f and the fitted quality of results was made in term of chi-square value obtained that is lower than 0.001.

![Figure 2](image)

**Figure 2.** (a) The Nyquist representation of Electrochemical Impedance Spectroscopy diagrams recorded for SS304L immersed in (1) Ringer, (2) Ringer + 10 g/L LA and low frequency range (b) for: (3) Ringer + 10 g/L H$_2$O$_2$ and (4) Ringer + 10 g/L LA + 10 g/L H$_2$O$_2$. The experimental data are represented in the graph by the symbol and the fitted results are represented by the continuous line.
The EIS diagrams in the Nyquist representation of the SS304L tested in all solutions used are presented in Figure 2. The increase of the semicircle arc (the semicircle didn’t intersect the important axis (X) named (Real Z) of Nyquist representation of Electrochemical Impedance Spectroscopy diagram for SS304L tested in solution Ringer’s and Ringer + 10 g/L LA, this behaviour is attributed to the frequency dispersion) denotes an increase in the stability of the passive film and a decrease of that radius indicates a decreasing of the resistance of the passive film.

To fit the Electrochemical Impedance Spectroscopy diagrams register for SS304L tested in Ringer, Ringer + 10 g/L LA, Ringer + 10 g/L H₂O₂ and Ringer + 10 g/L LA + 10 g/L H₂O₂ it was used a circuit equivalent complex [8] and the result of Rp values from fitted spectra are given in Table 3.

| Nr. | Sample                                | Polarization resistance [kohm·cm²] |
|-----|---------------------------------------|-----------------------------------|
| 1   | 304L stainless steel immersed in Ringer solution | 4.473E11                          |
| 2   | 304L stainless steel immersed in Ringer + 10 g/L LA | 1.473E11                          |
| 3   | 304L stainless steel immersed in Ringer + 10 g/L H₂O₂ | 1850                              |
| 4   | 304L stainless steel immersed in Ringer + 10 g/L LA + 10 g/L H₂O₂ | 825                               |

Electrochemical impedance spectroscopy can be represented as well in Bode diagram such as figure 3a logarithm of modulus |Z| vs. logarithm of (f) and Figure 3b logarithm of (f) vs. phase angle.

**Figure 3.** Development of EIS in Bode graph for: (1) Ringer, (2) Ringer + 10 g/L LA and low frequency range (b) for: (3) Ringer + 10 g/L H₂O₂ and (4) Ringer + 10 g/L LA + 10 g/L H₂O₂. (a) |Z| Modulus vs. (f) ; (b) Phase angle vs (f).

An advantage of bode diagrams is the ability to observe the impedance variation at high frequencies, which is generally omitted in the Nyquist representation. High impedance values attain at small frequency and middle frequency disclose a down reactivity or a good corrosion behavior for SS304L stainless steel indicates the presence of protective oxide film.

From Figure 3(b) it can be noted that the SS304L tested in Ringer’s solution layer display an almost capacitive reply, illustrated by a phase angle near to - 82° on a wide range of frequency. This
behaviour also show a distinctive behaviour of a small oxide protective film form on the surface of stainless steel immersed in Ringer solution.

3.3. Optical Microscopy investigations before and after corrosion
In Figure 4 the surfaces of SS304L was investigated ahead and after corrosion assay in Ringer solution, Ringer + 10 g/L LA, Ringer + 10 g/L H₂O₂ and Ringer + 10 g/L LA + 10 g/L H₂O₂ in order to investigate the corrosive effects.

![Figure 4](image)

**Figure 4.** Optical microscopy of SS304L (a) ahead corrosion, (b) after corrosion in Ringer solution, (c) Ringer + 10 g/L LA, (d) Ringer + 10 g/L H₂O₂ and (e) Ringer + 10 g/L LA + 10 g/L H₂O₂.

In Figure 4a it can be notice that the SS304L before corrosion have a rough surface without surface defects. After corrosion in Ringer solution (Figure 4 (b)) and 4 (c) after corrosion in Ringer solution + 10 g/L LA, 304L the surface of stainless steel there are less affected by pitting corrosion.

With the addition of 10 g/L H₂O₂ in the Ringer solution it is observed that on the surface of the 304L stainless steel (Figure 4 (d)) general corrosion occurs on the surface of the material while on the surface of the SS304L immersed in Ringer saliva with 10 g/L LA + 10 g/L H₂O₂ (Figure 4 (e)) a pitting corrosion is observed which covering almost the entire surface of the material.
These results obtained by optical microscopy are in well accordance with the results obtained by the electrochemical methods respectively: OCP and EIS.

4. Conclusions
The purpose of this research study is to assess the influence of lactic acid and reactive oxygen species ($H_2O_2$) and the synergistic influence of $C_3H_6O_3$ and $H_2O_2$ in Ringer solution, on the corrosion resistance of SS304L by electrochemical investigations.

From the OCP measurements it can be observed that with the addition of 10 g/L Lactic acid the open circuit potential value slightly decreases as compared to Ringer solution. This behavior is possible due to the low pH of solution with addition of lactic acid which affects the dissolution of stainless steel. When in Ringer solution is added 10 g/L $H_2O_2$ it can be noted that is an increasing of the potential value as compare with Ringer solution and with the solution containing 10 g/L lactic acid. This behavior is possible due to the oxidizing effect of $H_2O_2$.

In the case of Ringer solution with addition of 10 g/L LA + 10 g/L $H_2O_2$ in combination, there is an increase in the potential compared to the Ringer solution and compared with Ringer solution with lactic acid, but there is a decrease in the potential compared to the solution with 10 g/L $H_2O_2$. This behavior is also dependent on the fact that in the presence of $C_3H_6O_3$ (lactic acid (LA)) and $H_2O_2$ there was a decrease in pH.

Through the EIS methods can be deduce that with the addition of lactic acid and hydrogen peroxide and these two compounds in combination present in Ringer solution the $Rp$ value of SS304L decrease as compared with SS304L tested in Ringer electrolyte.

From the optical microscopy investigations of SS304L surfaces before and after corrosion in Ringer saliva and Ringer + 10 g/L LA (lactic acid) are the less attacked by pitting corrosion. When in Ringer solution is added 10 g/L $H_2O_2$ it can be seen that on the surface of the SS304L appear a general corrosion on the surface of the stainless steel while on the surface of the SS304L put in Ringer solution with 10 g/L Lactic acid + 10 g/L $H_2O_2$ a pitting corrosion is observed which covering almost the entire surface of the SS304L.

5. References
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