Stainless steel implantation-induced changes in surface characteristics, corrosion resistance and hemato-biochemical parameters of male rat

Sahar A.Fadl-allah[1, 3 *], Q. Mohsen[1] and Nahla S. El-Shenawy[2, 4]

[1]Materials and Corrosion Lab (MCL), Faculty of Science, Taif University, Taif, K.S.A
[2]Zoology Department, Faculty of Science, Taif University, Taif, K.S.A
[3]Chemistry Department, Faculty of Science, Cairo University, Giza, Egypt
[4]Zoology Department, Faculty of Science, Suez Canal University, Ismailia, Egypt
saharfadalla@hotmail.com

Abstract: In this study the physiological solution effect on corrosion resistance and surface characteristics of stainless steel has been studied *in vitro* by electrochemical measurements and microstructure characterization of the surface. All studies were carried out using phosphate buffer saline (PBS) as a simulated physiological solution. Potentiodynamic polarization results indicated a considerable shift of pitting potential of the specimen in the noble direction after14 days of immersion in PBS. As evidenced by electrochemical impedance spectroscopy (EIS), the effect of long immersion of stainless steel in physiological solution on the passive film stability was proved. The surface structure and composition before and after immersion in PBS were then characterized by means of scanning electron microscopy (SEM) with electron diffraction X-ray analysis (EDX) techniques. The electrochemical measurements and fitting parameters showed that the passive film formed on stainless steel decreased the corrosion currents densities (I_{corr}) and the constant phase elements (*CPE*), as simultaneously increased the values of polarization or charge transfer resistance (R_{ct}) of stainless steel in simulated physiological solution. The physiological and histological effects of pitting corrosion of stainless steel metal were studied after 14 days of post-implantation in the tibiae of Sprague-Dawley male rats. The stainless steel implantation caused a slightly increased in blood haemoglobin, total erythrocytes count and packed cell volume, and significantly decreased total leukocyte count. All the hepatic enzymes activities of a separate aminotransferase, alanine aminotransferase, alkaline phosphatase and lactate dehydrogenase were significantly decreased. The activity of glutathione S-transferase and the level of lipid peroxidation were significantly increased while hepatic glutathione was significantly decreased. The toxicity of stainless steel in implanted rat could be related to the biodegradation of the alloy and releasing of Fe, Mn, Ni and Cr in the rat tissue as indicated by the *in vitro* study. The bone regeneration was observed at the surface near the stainless steels implants after two weeks of implantation.

[Sahar A.Fadl-allah, Q. Mohsen and Nahla S. El-Shenawy. **Stainless steel implantation-induced changes in surface characteristics, corrosion resistance and hemato-biochemical parameters of male rat**. Journal of American Science 2011;7(1):84-91]. (ISSN: 1545-1003). http://www.americanscience.org.

***Keywords*:** Impedance spectra; Pitting corrosion; Scanning electron microscope (SEM); Electron diffraction X-ray (EDX) analysis; Lipid peroxidation; Glutathione; Toxicity; Bone repair.

1. Introduction

Biocompatibility is the ability of a material to perform with an appropriate host response in a specific application. This means that the tissue of the patient that comes into contact with the materials does not suffer from any toxic, irritating, inflammatory, allergic, mutagenic, or carcinogenetic action. Hence the attention of researchers to study the corrosion susceptibility of various metals and alloys used in surgical implantation in the physiological fluids (Baroux, 1993). Surgical implants are usually made of metallic materials, such as titanium and its alloys, stainless steels and cobalt - chromium alloys. Among all the metallic materials, stainless steel is the most popular because of their relatively low cost, ease of fabrication and reasonable corrosion resistance. However, stainless steel is susceptible to a number of localized corrosion, such as pitting and crevice corrosion, intergranular corrosion (IGC) and stress corrosion cracking (SCC) (Shaikh et al., 2006). A number of failures of stainless steel materials during its implantation have been reported (Rondelli et al., 2005); due to their high nickel (Ni) content and to the aggressive biological effects. The corrosion products include iron, chromium, nickel and molybdenum. Although new Ni-free stainless steels has been subjected to biological studies and shown promising results (Fini et al., 2003) until now many of the developing countries still use commercial stainless steel which contains Ni element particularly in the filed of bone surgeries. Stainless steel implants are used as temporary implants to help bone healing, as well as fixed implants such as for artificial joints. Typical temporary applications are plates, medullar nails, screws, pins, sutures and steel threads and networks used in fixing fractures (Virtanen et al., 2008). Although stainless steel is seldom used in developed countries as permanent implants, it is still the most used in emerging countries (Ballarre et al., 2010). Stainless steels are iron-base alloys with a minimum of 10.5% Cr as an alloying element, needed to prevent the formation of rust (Haritopoulos et al., 2007). The susceptibility of stainless steel to the different types of corrosion, especially pitting corrosion depends primarily on the environmental parameters besides the chemical composition and metallurgical manufacturing condition of the steels. The effects of various anions present in surrounding environment on the pitting of stainless steel have been studied by many authors. Zuo et al (2002); reported the inhibition effects of OH^-, NO^{3-}, SO_4^{2--}, ClO_4^- and acetate ions on pitting of stainless steel in chloride solutions. An increase of Cr content strongly increases the resistance against localized breakdown of passivity. Reliable prediction of the corrosion behaviour is the fundamental step towards effective control of corrosion. Electrochemical measurements involving electrochemical polarization and electrochemical impedance spectroscopy techniques were performed in physiological solutions in order to determine and compare the corrosion behaviour of the different implanted materials under variable conditions (Hiromoto et al., 2002). Electrochemical polarization methods are classified as controlled potential (potentiostatic, potentiodynamic) and controlled current (galvanostatic) approaches. The polarization curve associated with a potentiodynamic method allows detailed study of the important parameters that impact the formation and growth of passive films (E_{corr}) and pit propagation (E_{pit}). This method was successfully used to explain the pitting and passivation on stainless steel. On another hand, EIS has been successfully used to investigate the corrosion and passivation phenomena (Fadl-Allah et al., 2008). It enables the direct matching of the electrochemical system to equivalent circuit models. These equivalent circuits consist of discrete electronic components, resistor, capacitors and/or inductors, which can describe the properties of the electrochemical system under investigation.

Despite the high corrosion resistance of stainless steels and good mechanical properties, but corrosion occurs promoting the release of metal ions that penetrate into the biological tissues. The ions that released are disseminated throughout the body and partially accumulated in the liver, kidneys and spleen. Surface characterization of these metallic alloys is highly important as a tool to evaluate the performance of the implant through the interaction surface film-tissue and the possible migration of metallic ions from the base metal to the nearby tissue. The analysis of *in vivo* formation of new tissue at the interfaces of bioactive implants has been reported using histological methods and the interfacial mechanical.

Therefore, the present study was carried out *in vitro* to (1) provide an improved understanding of the corrosion process on stainless steel when soaked in simulated physiological solution which is prepared by dissolving only inorganic components, (2) characterize the relationships between corrosion behaviour and surface characteristics of stainless steel before and after two weeks from its immersion in simulated physiological solution by using scanning electron microscopy (SEM) to study the surface morphology and electron diffraction X-ray analysis (EDX) to analyze the chemical composition of the surface. Moreover, the aim of this study was to evaluate physiological and histological effect of pitting corrosion of local stainless steel metal in osteosynthesis of the body. Some haematological parameters and the alterations in the levels of glutathione, lipid peroxidation and some enzyme activities of liver tissues of male rat were determined after two weeks of post-implantation of commercially stainless steel laminar in tibiae of rat.

2. Materials and methods

2.1 Materials and Chemicals

The implants used in this study were stainless steel with the chemical composition of the metal that is shown as follows: Cr: 19.22%, Ni: 7.8%, Mn: 1.2%, Si: 0.5%, C: 0.019% and Fe: Balance, see Table 1. Samples of stainless steel for electrochemical measurements were machined down to 1 mm in diameter, 3 mm in width and approximately 6 mm in length. They were polished with different grit emery papers up to 4/0 grade, cleaned with distilled water and rinsed in ethanol before mounted in an electrochemical cell. The sample was partially immersed to a constant depth in the testing solution during the experiments. Testing solution of phosphate buffer saline (PBS) [8.77g dm^{-3} sodium chloride (NaCl), 1.42 g dm^{-3} di-sodium hydrogen phosphate (Na_2HPO_4) and 2.72 g dm^{-3} potassium di-hydrogen phosphate (KH_2PO_4)] was prepared from analytical grade reagents and triply distilled water. The test solution was adjusted at pH = 7.4. This test solution was chosen to simulate the physiological solution in order to be able to compare the *in vitro* results with the *in vivo* data.

Table 1**.** Chemical compositions (in wt%) of the stainless steel samples before (blank) and after immersion in physiological solution (PBS).

Sample	O	Si	Fe	Mn	Ni	Cr	C	Na	P	K	Cl
Blank	-	0.50	68.13	1.22	7.82	19.67	3.11	-	-	-	-
Immersed for 14 days	16.67	-	40.17	0.77	4.34	10.87	7.31	8.19	5.41	1.33	4.94

2.2 *In vitro* experimental and analysis

Electrochemical and Corrosion test

The samples were immersed in simulated solution (PBS) during 14 days. All electrochemical measurements were accomplished with an Autolab (PGSTAT30 with GPES and FRA modules, Ecochemie) in a one compartment three-electrode cell where a platinum wire counter electrode (CE) and a saturated calomel electrode (SCE) as reference to which all potentials are referred. The working electrode (WE) was in the form of a plate cut where the exposed surface areas of the investigated materials was 0.16 cm^2. The potentiodynamic current - potential curves were recorded by changing the electrode potential automatically from – 800 mV to + 2500 mV, just after exposition to the electrolyte solution. The potential scan rate was 1 mV/s. Corrosion current densities (I_{corr}) and corrosion potential (E_{corr}) were evaluated from the intersection of the linear anodic and cathodic branches of the potentiodynamic curve as Tafel plots. Electrochemical impedance spectroscopy (EIS) is a non- destructive sensitive technique which enables the detection of any changes occurring at the electrode/electrolyte interface. Impedance data were presented as Bode plots. Bode plots are recommended as standard impedance plots, since all impedance data are equally represented and the phase angle, θ, is a sensitive parameter for any surface changes. All EIS spectra were acquired by applying the open circuit potential over a frequency range of 10^{-1}–10^5 Hz to evaluate the structure stability of stainless steel in PBS. Samples were tested at two periods of immersion time. The results were analyzed using the fit program FRA (Fadl-Allah and Mohsen, 2010). Before impedance or polarization measurements, the working electrodes were immersed in the test solution until a steady state of the open-circuit potential was reached. Each experiment was performed at least twice with a new surface for each run.

Microstructure characterization of surface

Before the polarization experiments, the scanning electron microscope (SEM) photographs were carried out for stainless steel samples to study the morphology of samples before and after immersion in the physiological solution, using SEM Model Philips XL 30 attached with EDX Unit and accelerating voltage 30 kV., magnifications from 1500X up to 15.000X. Samples were coated with a thin layer of gold to prevent charge problem and enhance the resolution. The composition of the surface film, before and after immersion of the samples in physiological solution, was characterized by EDX analysis.

2.3 In *vivo* experiments and analysis

Experimental animals and Implantation

Wistar rats weighing 90-100 g (n = 10) were purchased from King Fahed Medical Research Centre in Jeddah (Kingdom of Saudi Arabia). They were acclimatized and fed *ad libitum* with rodent chow and tap water for a minimum of seven days before surgical process. Animals were randomly divided into two groups with five animals in each; control group and laminar implants group. The European Community Directive (86/609/EEC) and National rules on animal care have been followed.

The animals were anesthetized intraperitoneally with a solution of 8 mg ketamine chlorlhydrate and 1.28 mg xylazine per 100 g body weight. The skin of right tibiae was shaved before a 1.5 cm incision was made along the tibial crest. The region of surgery surface was cleaned with antiseptic. The subcutaneous tissue, muscles and ligaments were dissected to expose the lateral external surface of the diaphyseal bone. An end-cutting bur was used to drill a hole 1.5 mm in

diameter with manual rotating movements to avoid overheating and necrosis of the bone tissue http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6WGW-4SXRTPT-1&_user=5652352&_coverDate=11%2F30%2F2008&_alid=828697825&_rdoc=54&_fmt=full&_orig=search&_cdi=6833&_docanchor=&view=c&_ct=3726&_acct=C000067180&_version=1&_urlVersion=0&_userid=5652352&md5=e509aa5caae1144ebfda1c14e0052f0a#bib7 (Cabrini et al., 1993). No cooling with NaCl was required. No antibiotic therapy was administered. Laminar implants of commercially stainless steel implants of 3.0 × 1.0 × 1.0 mm exhibited a predominantly smooth surface with irregularities that are characteristic of the lamination process. After 14 day of post-implantation, the blood samples were collected from animals in the tube containing ethylenediaminetetraacetic acids (EDTA) under light anaesthesia for blood analysis.

Haematological and Biochemical parameters

Blood samples were collected from the retro-orbital plexus vein of the animals according to Schermer. Blood samples were transferred to test tubes containing EDTA for haematological parameters [red blood cell (RBC) counts, haemoglobin (Hb), packed cells volume (PCV), white blood cell (WBC) counts, lymphocytes counts, mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and thrombocytes] using Systemax KX21N haematology analyzer. Each sample was run in duplicate.

Liver was removed from rat under ether anaesthesia after 14 day of implantation and washed with cold saline buffer. Washed tissues were immediately stored at -80 ºC. To obtain the enzymatic extract, tissues were homogenized in ice cold 50 mM sodium phosphate buffer (pH 7.0) containing 0.1 mM EDTA to yield 10% (W/V) homogenate. The homogenates were then centrifuged at 4000 rpm for 10 min at 4 ºC. The supernatant were separated and used for determination of enzymes activity of alanine aminotransferase (ALT), a separate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and glutathione-S-transferase (GST). The data expressed in international units per gram (IU/g). These biomarkers for liver damage were determined using UV kinetics methodology of the commercial diagnostic kit (Stanbio Co., Spain). Total protein was determined using bovine serum albumin (BSA) as standard and values were expressed as mg/g.

The lipid peroxidation (LPO) was estimated as the concentration of thiobarbituric acid reactive product malondialdehyde (MDA) by using the method of Ohkawa et al. (1979). It was measured spectrophotometrically at 532 nm by using 1,1,3,3-tetraethoxypropane as an external standard. LPO was expressed as MDA in µmol/g of liver tissue. Glutathione (GSH) was measured in tissue homogenates of liver after reaction with 5, 5′-dithiobis-(2-nitrobenzoic acid) using the method of Beutler et al. (1969). The GSH content was expressed as mM GSH/g tissue using a calibration curve prepared by known concentrations of reduced glutathione.

Histopathology

Histopathological examination was carried out according to Drury and Wallington (1980) at the end of the experiment. The animals were killed by ether overdose; the tibiae were removed and fixed in 10% formalin solution for 14–18 h, EDTA solution is used to decalcify bone specimens for histological examination. Then, the samples passed in a series of graded ethanol and embedded in paraffin. Paraffin sections were cut at 5 µm thickness by rotatory microtome and stained with haematoxylin and eosin (H & E) stain for light microscopic examination.

Statistical analysis

Statistical analysis was based on comparing the values between laminar implants group and control group. The results are expressed as means ± SD (n=5). Statistical comparisons were performed using One-way Analysis of Variance (ANOVA) using SPSS statistical software package version 13. The level of significance was taken below $P < 0.05$.

Table 2. Electrochemical parameters calculated from the potentiodynamic polarization curves at different times of immersion of stainless steel samples in physiological solution (PBS).

Immersion time	E_{corr} / mV_{SCE}	E_{pitt} / mV_{SCE}	I_{corr} / A cm^{-2}	I_{pass} / A cm^{-2}
Zero time	-300	300	8×10^{-7}	6×10^{-2}
1 day	-20	650	2×10^{-8}	4×10^{-2}
14 days	-250	800	2×10^{-8}	2×10^{-3}

3. Results

3.1 *In vitro* electrochemical measurements

Potentiodynamic polarization

The potentiodynamic polarization technique was used to investigate the electrochemical behaviour of stainless steel in physiological solution, PBS. Representative polarization curves from the potentiodynamic polarization measurements are displayed in Figure. 1. The quantitative corrosion values of corrosion potential (E_{corr}), corrosion current density (I_{corr}), passivation current density (I_{pass}) and pitting potential (E_{pit}) obtained through the polarization curves were calculated and are presented in Table 2. The greatest negative E_{corr} value of -300 mV was observed for the specimen just immersed in PBS. The E_{corr} of specimen was shifted in the noble direction with the time of immersion in PBS. Hysteresis through potential ranging from -300 to 600 mV was found for all stainless steel samples in PBS. These results refer to the passivity is not stable, and indicative of pitting corrosion.

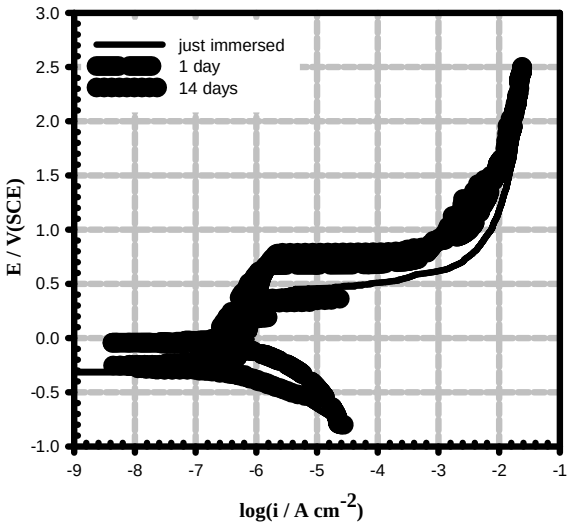


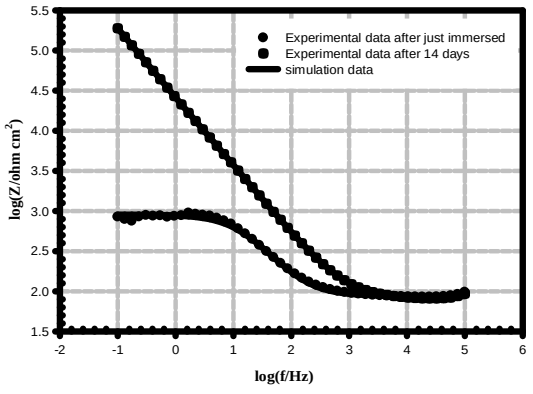
Figure. 1. Polarization curves obtained for Stainless Steel after different times of immersion in phosphate buffer saline (PBS) at pH 7.4.

Impedance measurements

The impedance results for the stainless steel in physiological solution after immersion for two different times are shown in Figure 2. These spectra indicate that the long immersion time of samples in PBS plays an important role to change the properties of the passive film formed on samples. The Bode plots recorded for the stainless steel specimens after zero time (just

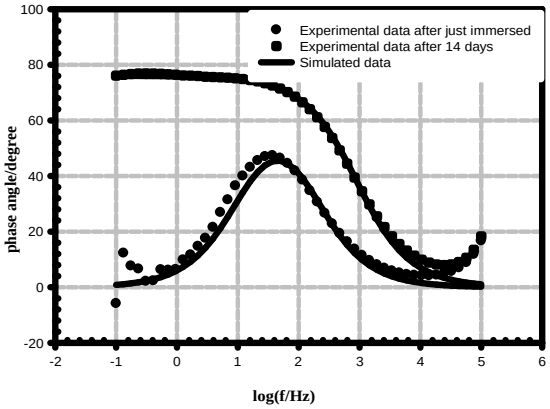
immersed) and two weeks of immersion in PBS are presented in Figure 2a and 2b respectively. The impedance bode plot (Figure 2a) show that, the impedance resistance of the specimen change with the time of immersion. It is noted that, the impedance values at the minimum frequency range, which correspond to the corrosion resistance of the electrode material, increases with increases the time of specimen immersion in PBS. On the other hand, the phase bode plot (Figure 2b) show that only one phase maximum, which suggests that the time constant of the protective film RC circuit is much greater than that of the double layer RC circuit. This result refer to the corrosion process was mainly charge transfer controlled. The general shape of the curves is similar for all the stainless steel specimens, indicating that almost no change in the corrosion mechanism occurred due to the immersion time (Rosliza et al., 2008).

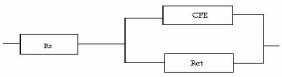
Another parameters related to impedance analysis derived by curve fitting method are summarized in Table 3. Generally, the equivalent circuit model is representing the surface properties of stainless steel specimen in PBS solution and it considers a good way that can be proposed to simulate the experimental results appropriately. Figures 2a and 2b show the computer fitted values and experimental impedance data of specimen in PBS. Charge transfer resistance R_{ct} and capacitance C were obtained by fitting the spectra (0.1-1000Hz) using a simple equivalent mode (Figure 2c). The results showed that R_{ct} values increased with increasing immersion time, the capacitance, C values decrease indicating the formation of a surface film.



(a)

(b)





(c)

Figure. 2. Impedance data recorded for stainless steel at different immersion times in phosphat buffer saline (PBS) at pH 7.4. (a) Bode-impedance pots, (b) Bode-phase plots, (c) Equivalent circuit used for fitting experimental impedance data where R_s is the solution resistance, R_{ct} is the charge transfer resistance, and CPE is a constant phase elements.

Table 3**.** Polarization resistance values are calculated by EIS measurements at different time of immersion of stainless steel samples in physiological solution (PBS).

n	*CPE*/μF cm^{-2}	R_{ct} / KΩ cm^2	R_s / Ω	Immersion time

11/26/2010