

# Degradation of implantable materials – in vivo and in vitro research

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## Abstract

The article concerns the biological and electrochemical degradation of metallic implants in vivo and in vitro studies. The in vivo research dealt with degradation of plates used to join bones, as well as endoprostheses. The most common damages were: metalosis, breaking in the microstructure changes, breaking in area of holes, as well as plastic deformation throughout the length of an implant. The material used for the research was pure titanium. The analysis of the reasons of the damages included the observation of the surface scrap, which was conducted by the use of Philips XL30 electron scanning microscope. In vitro studies concerned the impact of aggressive environments on the external surface of implants. The titanium pins were immersed in the bacterial solution and corrosion tests were carried out in two solutions simulating the human body (Ringer's and Artificial Saliva). It was found that after a period of 6 months, the bacteria began to settle on the surface. As a results of the electrochemical corrosion processes: general etching, appearance of micropores and intensive-ly corroded areas were observed on the surface. Based on in vivo and in vitro studies, the susceptibility of titanium implants to degradation in the human body is assumed.

**European Journal  
of Medical Technologies**  
2016; 4(13): 38-46

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www.medical-technologies.eu  
Published online 30.12.2016

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## Key words:

degradation, metallic  
implants, titanium,  
corrosion, biofilm.

## Introduction

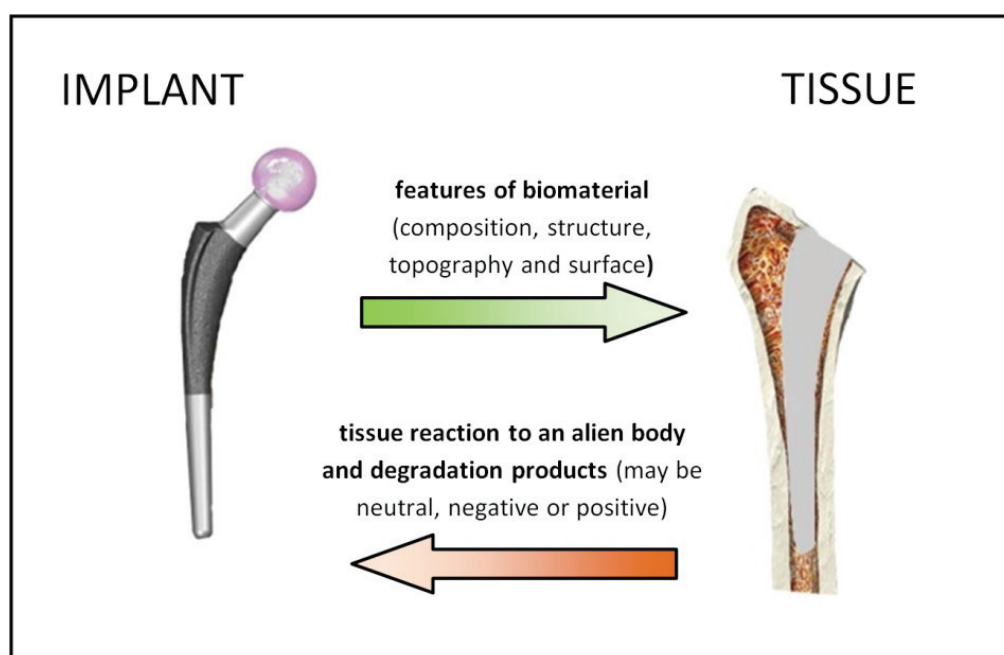
The most important thing in clinical implantology is biotolerance. Cooperation between an implant and a tissue is two-way interface. It includes the impact of an implant on a tissue and the reaction of the tissues to an artificial item or the products of its degradation (Fig. 1) [1-3].

An implant introduced into the body is subjected to the influence of all the factors, which it will have to cope with throughout the whole period of staying in the body. Introducing an implant into any organ of the body needs an operation, which results in damaging some soft tissues and it may also cause, that some microorganisms may get into the body and later on result in inflammation. Therefore it would be expected from the implant not to cause undesirable reactions, but also to be able to destroy the pathogens at the edge of implant-tissue [1,3,4].

Introducing the metal biomaterials into the body is followed by forming the passive layer and covering an implant with proteins and body liquids on its surface. The proteins have an impact on various processes, for ex. sticking bacteria to the implant, which is consequence leads to biofilm formation. The biofilm is a complex structure including multi cell bacteria surrounded by organic and non organic layer. They are generated by microorganisms, which tend to show

adhesion to biological and abiotic surfaces. Forming biofilm is the reaction of microorganisms to the surrounding conditions, which determine their existence. Infections connected with biofilms are usually protracted and bacteria, which are responsible for its forming are resistant to drugs (Fig. 2) [4-8].

Another problem connected with the application of implants is oversensitiveness of alive tissues to the elements contained in metal implants. Surgical treatment of fractures involves indirect invasion into trauma environment, which has an influence on natural course of bone healing. According to literature concerning surgical treatment of shaft of femur fractures with adults there is possibility of local complications, despite applying different osteosynthesis methods and having good treatment results [4,9-11]. To avoid complications the least invasive method of surgical treatment should be chosen. The healing process of fracture ought to be similar to natural one [12]. The choice of the most infective method depends on different factors. The kind of fracture, the age and general condition of a patient, the condition of soft tissue surrounding the damaged bone, the set of implants. The surgical experience of the team is of paramount importance. Intramedullary osteosynthesis is the method which gives big possibility of effective operation, with relatively rare occurrences of local and general complications [12,13].



**Fig. 1.**  
The interaction between implant-tissue

Before being produced every implant is subjected to many laboratory examinations, which define the implants ability to transfer the loading for relatively long time. While performing the examination on the machines imitating the movements of an implant in an organism the item is acted on by forces several times bigger than the ones in natural environment. Unfavourable impact of forces may cause early consumption or total destruction of the implant [6,14].

After introducing the biomaterial into the body, the passive layer builds up on its surface. The passive layer covers the whole surface and shouldn't be destroyed in any way. However, it sometimes may be broken. Than alive tissue is in direct contact with the implant, which results in getting the alloy components into tissue environment. Such situation may lead to inflammation and dying of cells, which are in contact with implant surface, which is followed by metalosis (tissue disease) [7-9,14].

The most frequently occurring problem connected with metallic materials is corrosion. Strongly degradational body environment [5,15,16], as well as high temperature and lowered pH promote this type of degradation. It may significantly limit long term performance of an implant. Occurrence of inflammation

symptoms may be the reason for the removal of an implant from the body.

This paper deals with biological and electrochemical degradation of implants. Biomaterials have been tested in vivo and in vitro. The results of the study were shown by photos and using scanning electron microscope Phillips XL30.

## IN VIVO DEGRADATION ON THE IMPLANT

The material, which was provided to analyses came from the human body, in which it was subjected to degradation, but it was not damaged by the patient (bad quality of the material, improper heat treatment) (Fig. 3-5).

Testing of samples obtained after removal from the living organism shows the variety of degradation.

Fig. 6 shows the area, where the tissue was damaged. Biofilm is visible throughout the endoprosthesis.

Another sample shows (Fig. 7) corrosive focus. The sample was taken from plate used for join bones. Cracks are visible on the sample. Single bacteria were observed (Fig. 8).



**Fig. 2.**

Biofilm. A sample cut from the implant titanium (Ti6Al4V, plate teaming the long bones) with visible biofilm [9]



**Fig. 3.**  
Endoprosthesis with metallosis



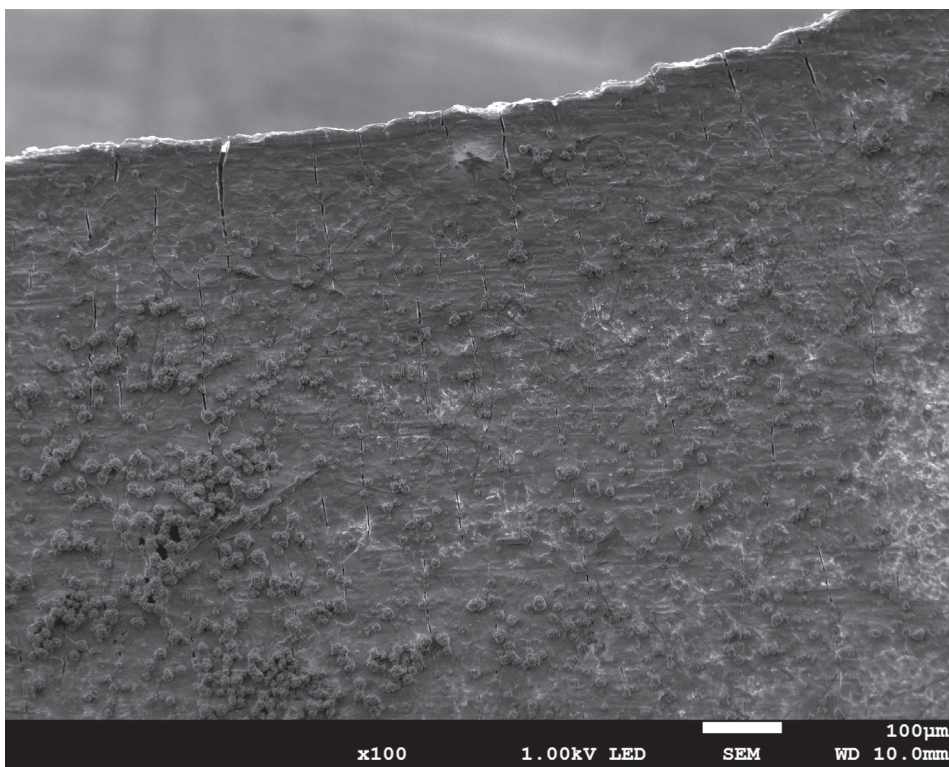
**Fig. 4.**  
Plates used for joint bones with corrosive focus



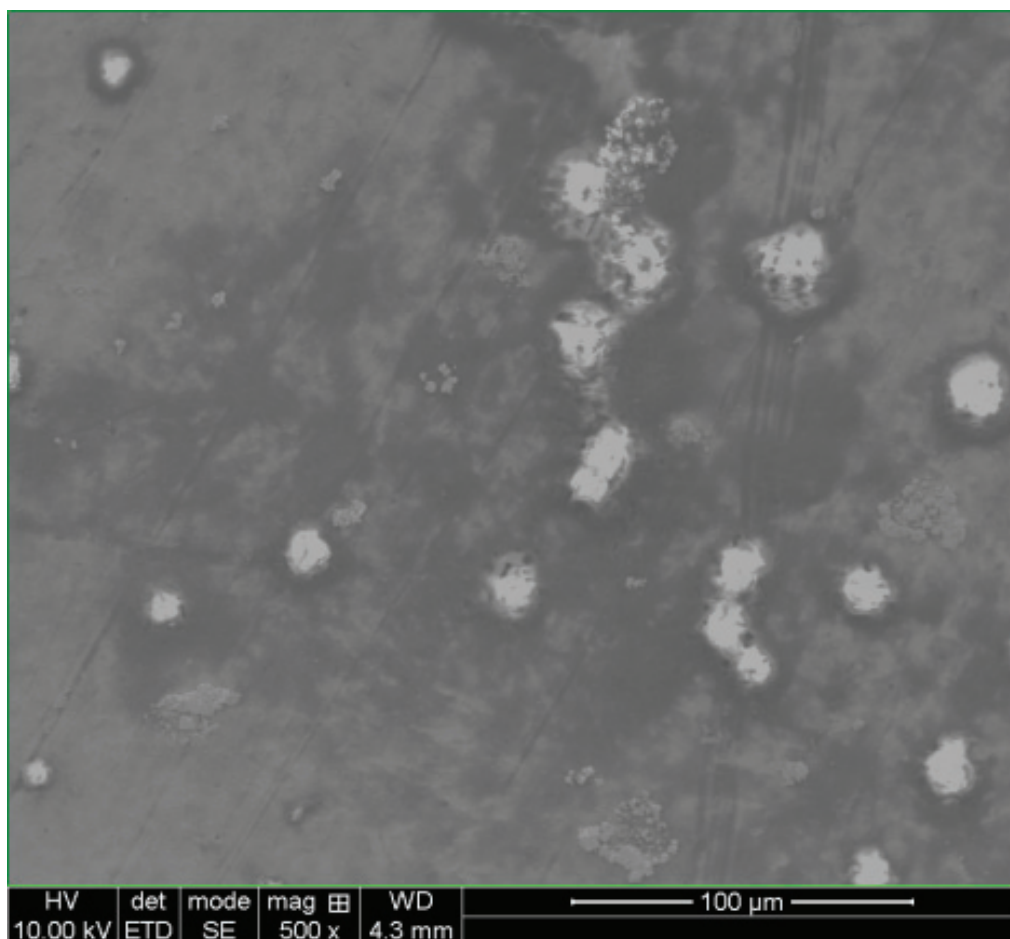
**Fig. 5.**  
Broken endoprosthesis



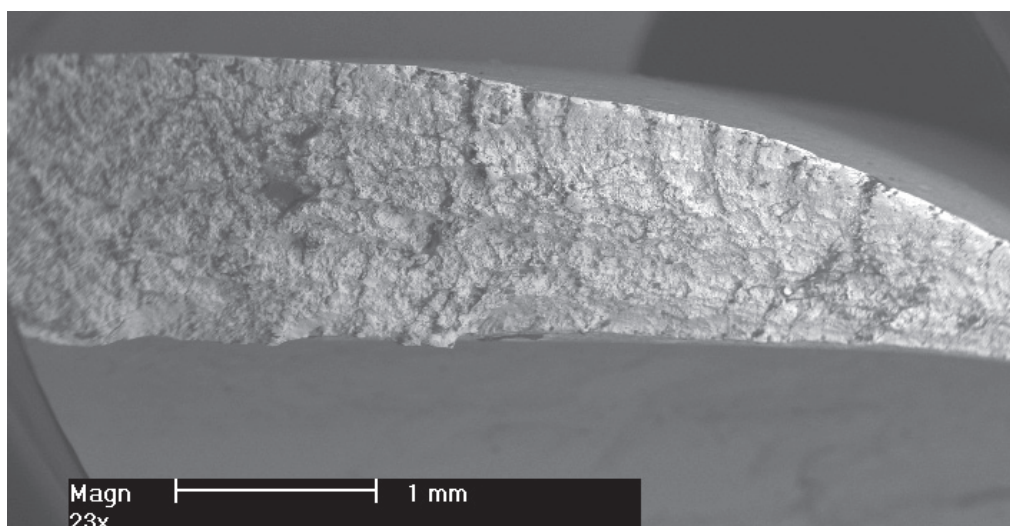
**Fig. 6.**  
Biofilm on the surface of the implant



**Fig. 7.**  
Cracks on plate used for join bones



**Fig. 8.**  
Single bacteria visible in the cracks



**Fig. 9.**  
Fatigue scarp on the sample

The third implant was removed from the living organism, because it broke. Fatigue scrap appeared on the sample (Fig. 9).

## In vitro degradation on the implant

On the basis of the knowledge acquired in the course of the research the bacteria solution consisting of five bacteria most frequently occurring in the operation area (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*) was created. The above mentioned solution was applied [P1] for further research connected with the degradation of implant materials.

The implants (plate used to join bones and endoprosthesis – new one) were cut into small pieces (15x15x5 mm). The specimens were dipped into bacteria liquid (the content as in Table 1) for the duration of 1, 3 and 6 months.

After 6 months in the bacterial solution, single bacteria were visible on the surface (Fig. 10).

At the same time, corrosion tests for samples were carried out as in the biological study. Corrosion test were performed on titanium specimens immersed in simulated body fluid solutions (SBF). The specimens before the study were: ground, skimmed and covered with an insulating resin were covered with an insulating resin. The area affected by corrosion was 10x10 mm (Fig. 11).

Specimens prior to the study were placed in SBF solutions for 24 hours at room temperature. There were two kind SBF solutions: Ringer solution (composition of purchased solution – Table 2) and Artificial

Saliva prepared by dissolving of the reagents according to Table 3 [17].

Three electrodes were used in the study: counter electrode (standard platinum electrode), reference electrode (Ag/AgCl saturated with potassium chloride KCl) and electrode discharged from the test sample. In the studies corrosion potential ( $E_{corr}$ ) and corrosion current density ( $I_{corr}$ ) were specified from the polarization curves by using the Tafel extrapolation method. The studies were conducted on potentiostat/galvanostat Atlas 0531 (Atlas Solich). The parameters were as follows: a potential change rate 1 mV/s, a scan range 600-2000 mV.

Based on the conducted research, it was found that for titanium specimens immersed in Ringer's solution average corrosion density  $I_{corr} = 51,1 \text{ nA/cm}^2$  and average corrosion potential  $E_{corr} = -373,3 \text{ mV}$ . While for titanium specimens immersed in Artificial Saliva solution:  $I_{corr} = 25,7 \text{ nA/cm}^2$  and  $E_{corr} = -437,1 \text{ mV}$ . Detailed results are shown in Table 4 and Table 5. Sample potentiodynamic polarization curves is shown on Fig. 12.

Corrosion test, however, affect to the structure of investigated materials. After the test, the samples were subjected to SEM microscopy.

As a result of corrosion tests on the outer surface of material notice a significant change in its structure (Fig. 13). By analyzing topography of the surface after corrosion, there is general surface deposition and the occurrence of micropores, and also the more intense corrosion areas (circles on Fig. 14).

Corrosion changes on the surface of titanium specimens (micropores – red circles and intense corrosion areas – green circles)

**Table 1.**

Chemical composition of the bacteria liquid

Component	Content (g/dm <sup>3</sup> )
Caseine peptone	17
Pepton S	3
NaCl	5
Na <sub>2</sub> HPO <sub>4</sub>	2.5
Glucose	2.5

**Table 2.**

Composition of Ringer solution at pH 5-7,5

Reagents	Content (g/L)
NaCl	8.6
KCl	0.3
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.33
NaOH	to set pH
HCl	To set pH

**Table 3.**

Composition of Artificial Saliva at pH 8.3 [17]

Reagents	Content (g/L)
$(\text{NH}_2)_2\text{CO}$	0.13
NaCl	0.7
$\text{NaHCO}_3$	1.5
$\text{Na}_2\text{HPO}_4$	0.26
$\text{K}_2\text{HPO}_4$	0.2
KSCN	0.33
KCl	1.2

**Fig. 10.**

Specimen after 6 months' staying in bacteria solution

**Fig. 11.**

Specimens for corrosion testing



**Table 4.**

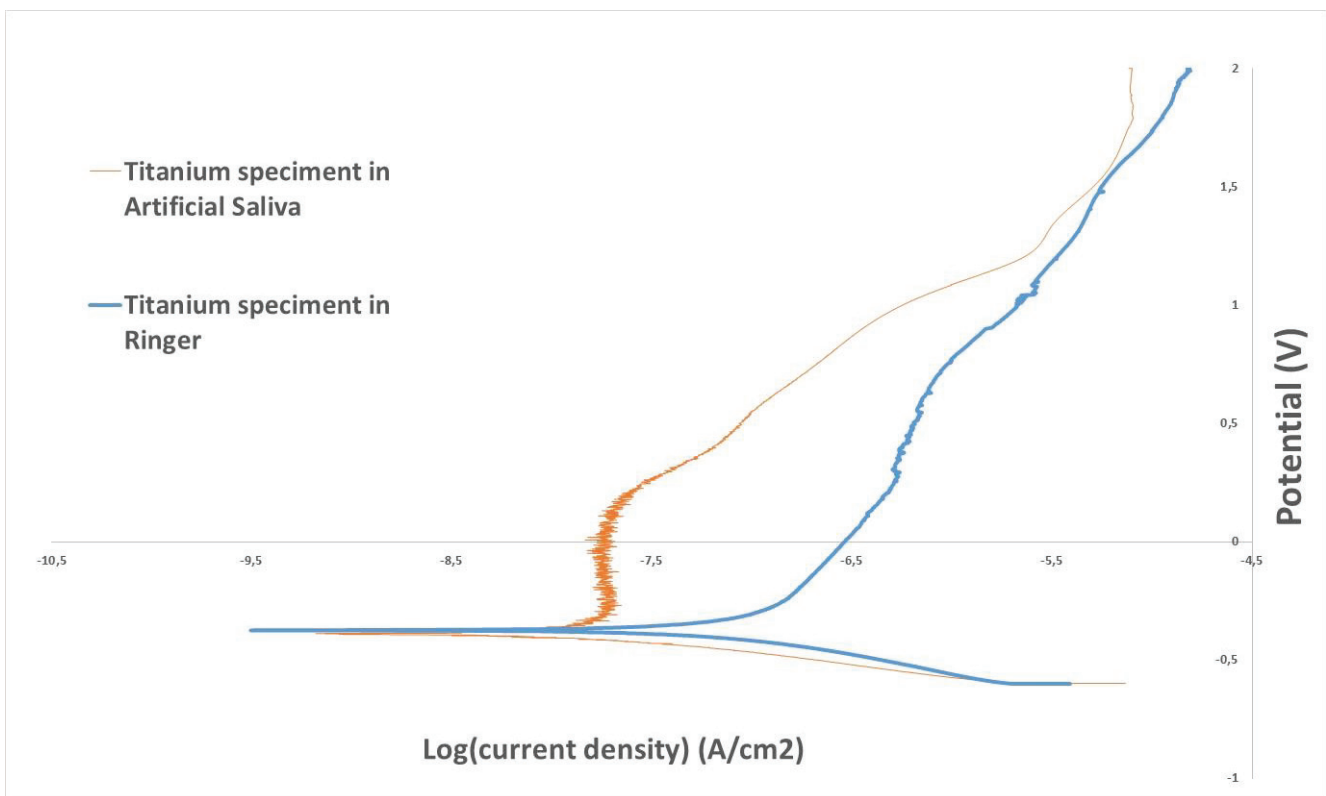
Corrosion test results in Ringer Solution

$i_{corr}$ [ $nA/cm^2$ ]	$E_{corr}$ [mV]
53,26	-392,91
64,45	-372,24
35,42	-354,85
$\Sigma 51,1$	$\Sigma -373,3$

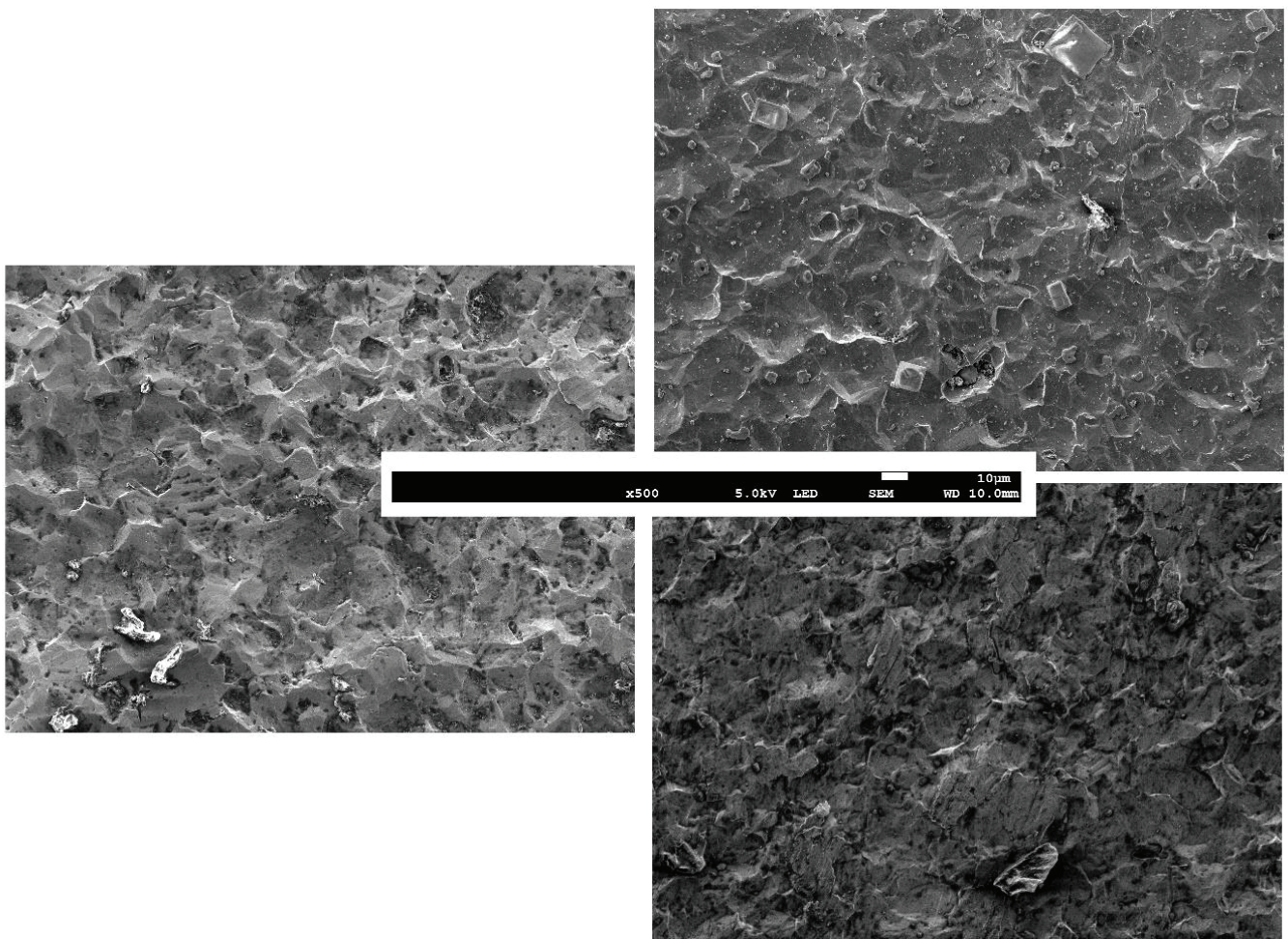
**Table 5.**

Corrosion test results in Artificial Saliva

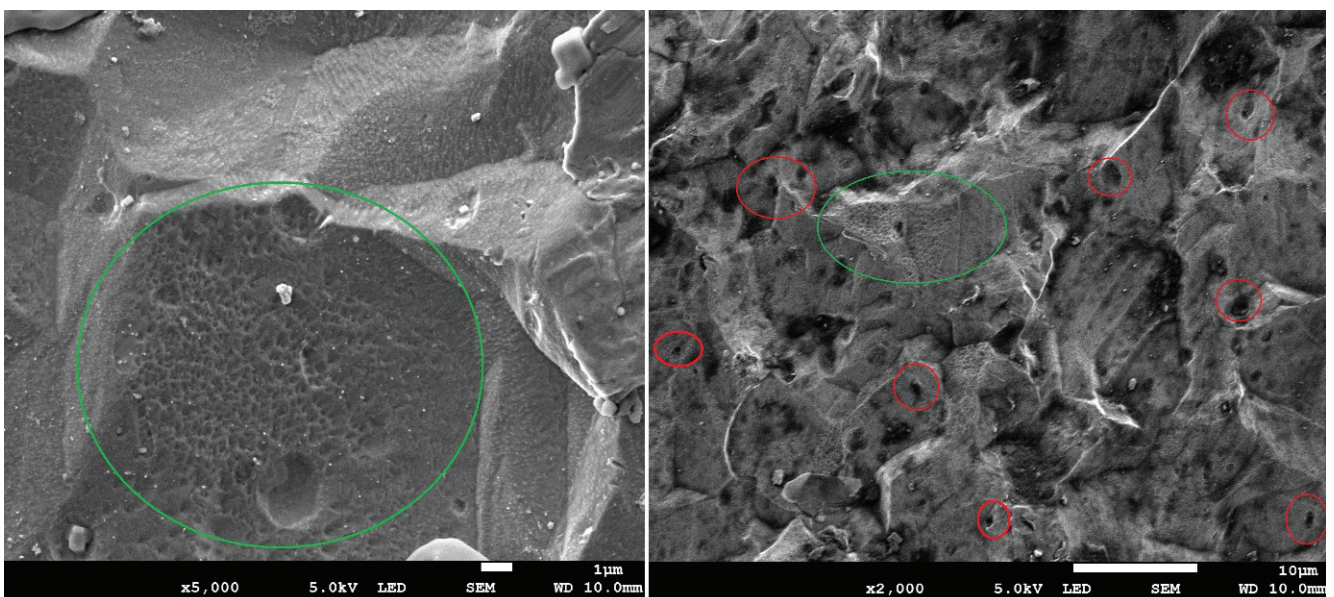
$i_{corr}$ [ $nA/cm^2$ ]	$E_{corr}$ [mV]
26,24	-386,94
21,37	-407,35
29,54	-418,44
$\Sigma 25,7$	$\Sigma -437,1$

**Fig. 12.**

Sample potentiodynamic polarization curves of tested specimens



**Fig. 13.** Topography of the surface of titanium before the corrosion test (left) and after corrosion test (right and Ringer – up, Artificial saliva – down)



**Fig. 14.** Corrosion changes on the surface of titanium specimens (micropores – red circles and intense corrosion areas – green circles)

## Discussion and conclusions

The main aim of an implant is performing the definite functions in the body – correction or repairment of certain organs. However, it may occur, that as a result of various factors an implant will not be capable of performing its task properly. The loss of functional features may result from: mechanical damages of an implant, post-operation complications - mainly inflammations and some reactions leading to the rejection of an implant. Therefore different modifications of the surface are in use: hydroxyapatite or nanometals, in order to protect the tissue from harmful effect of the material, as well as to protect the material from bacteria activity. Using various kinds of surfaces enables the process of the osteoblasts adhesion. Therefore the surface must be very carefully chosen bases for cell adhesion and antibacterial as well [18,19].

Titanium implants are exposed to degradation in the human body. The main cause are mechanical damage, but on the other hand implants are exposed to biological, chemical and electrochemical degradation. Bacteria can settle on the biomaterial surface and produce a biofilm structure. In the case of chemical degradation, the outer surface of the implant is etching as a result of surface chemical reactions. Similarly in case of electrochemical degradation – corrosion – the top layer is destroyed (i.e. etching, pore formation and local intense corrosion areas). Corrosion of metallic implants can lead to the occurrence of metallosis. As a result of any kind of degradation, implants functionality may be impaired and there is a danger of the patient's health.

The presence of the implant in the living organism stimulates many mechanisms to remove it, reacting like a foreign body. Contemporary biomaterials have a limited lifetime, without the ability to reconstruct, and should therefore be removed after performing their function. They always cause inflammation, but not always the same intensity.

Hence, it is important to conduct in vitro and in vivo studies to minimize the risk of problems with the use of implants and work on their modification to achieve maximum biocompatibility.

## References

1. Świczko-Żurek B.: The biological reactions on the implant – organism border. *Engineering of Biomaterials* 2007; 10: 43-44.
2. Award N.K., Edwards S.L., Morsi Y.S.: A review of TiO<sub>2</sub> NTs on Ti metal: Electrochemical synthesis, functionalization and potential use as bone implants, In *Materials Science and Engineering: C*. 2017; 76: 1401-1412.
3. Chen Q., Thouas A.G.: Metallic implant biomaterials, In *Materials Science and Engineering: R: Reports* 2015; 87: 1-57.
4. Świczko-Żurek B., Pałubicka A., Krzemiński M.: The reactions occurring on the implant as a result of contact with human tissue. *Engineering of Biomaterials* 2009; 12: 38-39.
5. Bartmański M., Świczko-Żurek B., Zieliński A.: Influence of Staphylococcus bacteria adhesion on the degradation of the Ti-6Al-4V alloy. *Sylwan* 2015; 159.
6. Matos A.O., Ricomini-Filho A.P., Beline T., Ogawa E.S., Costa-Oliveira B.E. et al.: Three-species biofilm model onto plasma-treated titanium implant surface, In *Colloids and Surfaces B: Biointerfaces* 2017; 152: 354-366.
7. Świczko-Żurek B.: The allergic and irritating reactions to metallic implants with trauma-orthopaedic patients. *International Journal of New Technology and Research* 2016; 2: 50-56.
8. Zieliński A., Świczko-Żurek B., Ossowska A.: Environmental degradation of Ti alloys in artificial saliva and a role of fluorides. *Materials Engineering*, 2 (2014) 225-231.
9. Supernak-Marczewska M., Świczko-Żurek B.: Reactions on the surface of the implant under the influence of biofilm. *Advances in Materials Science* 2010; 10: 5-11.
10. Świczko-Żurek B., Szumlański A., Zorn J.: Allergy to implant components: chromium, nickel, cobalt and titanium. *Advances in Materials Science* 2009; 9: 41-47.
11. Wójcik K., Nowak R., Wroński S., Wojciechowski P., Służalek M.: Estimation of post-operation treatment of the shaft of femur fraction. *Movement Organ Surgery, Polish Orthopedics* 1999; 64: 31-38.
12. Zorn J., Świczko-Żurek B.: Allergic reactions as a defense of the organism to the influence of

- implants components made of stainless steel. *Advances in Materials Science* 2010; 10: 48-54.
13. Fromme P., Blunn G.W., Aston W.J., Abdoola T., Koris J., Coathup M.J.: The effect of bone growth onto massive prostheses collars in protecting the implant from fracture, In *Medical Engineering & Physics* 2017; 41: 19-25.
  14. Marciniak J., Chrzanowski W., Krauze A.: *Intramedullary nail in osteosynthesis. Monograph, WPŚl., Gliwice* 2006.
  15. Nałęcz M., Błazewicz S., Stoch L., *Biomateriały Tom 4. Akademicka Oficyna Wydawnicza Exit, Warszawa* 2003.
  16. Demczuk A., Świczko-Żurek B., Ossowska A.: Corrosion resistance examinations of Ti6Al4V alloy with the use of potentiodynamic method in Ringer's and artificial saliva solutions. *Advances in Materials Science* 2011; 11: 35-42. P1 Patent No P 409082 B. Świczko-Żurek: The evaluation method of biodegradable metallic implants, the process of obtaining a solution for the evaluation of a bacterial biodegradation and metal implants bacterial composition to evaluate the biodegradability of metallic implants.
  17. Loch J., Krawiec H.: Corrosion behaviour of cobalt alloys in artificial saliva solution, *Arch. Foundry Eng* 2013; 13: 101–106.
  18. Zieliński A., Świczko-Żurek B., Sobieszczyk S.: Estimation of the expected effects of different surface modifications on mechanical, chemical and biological behavior of endoprotheses. *Engineering of Biomaterials* 2006; 9: 217-219.
  19. Świczko-Żurek B., Jażdżewska M., Zieliński A.: The phase composition of the surface layer of laser melted Ti-6Al-4V bioalloy. *Engineering of Biomaterials* 2006; 9: 221-223.