

# Experimental researches on biomaterial-tissue interface interactions

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The aim of this paper is to present several methods for investigation of the biomaterials-tissue interactions. These methods were used to analyze different experimental implants made from metallic biomaterials for orthopedic and dentistry use, with different morphological aspects. For obtaining best and representative results, we used optical microscopy, corrosion tests, in vitro tests in the conditions of implantation in rabbits.

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## 1. Introduction

The biocompatibility problem appears along with the recognition of the major differences between the living tissues and the artificial ones. Both from historical perspectives and from practical point of view, there are known different types of interaction between tissues and implant material. In any of these interactions, we can observe both positive and adverse effects. From another point of view, these effects can be considered as toxic or non-toxic. Such considerations are relatively poor according to their amount and less for their quantification on an absolute scale.

## 2. Experimental

From the experimental point of view, in order to obtain a relevant study regarding biomaterial-tissue interface interaction, we had to approach the subject from different perspectives. First, we had to select a biomaterial class to cover a major part of the materials used in implantology, and then we had to characterize them from the compositional and microstructural point of view.

For as much the corrosion still represents one of the main limitations of the metallic materials use in implantology, experimental researches have been made on their electrochemical corrosion resistance in artificial physiological suspensions media. Taking into account the results of the characterization from compositional, microstructural and corrosion resistance points of view, we carried on the researches on a different level, from a totally different perspective by doing a cytotoxicity (for *in vitro* interaction tests) tests and by implanting new metallic samples with different roughness prepared surfaces into rabbits. One of the main reasons for obtaining good results regarding biomaterial-tissue interactions is the use of an ESEM type scanning electron microscope, which offers the possibility of studying biomaterial tissue couples without special sample preparation and without damaging the interface between coupled elements.

## 3. Results and discussion

To cover in our studies a wide part of the metallic biomaterials the selected samples were: titanium, Ti6Al4V alloy type, CoCrMo alloy, and medical destination austenitic stainless steel. The investigations regarding the tested biomaterials chemical composition were made on 5 different fields, compared with the minimum and maximum values presented in ASTM standards for the studied metals and alloys. The samples were etched using specific chemical reagents for each material. Where was necessary the metallographic etching was made electrochemically. The 316L steel ranges within the ASTM F138 limits, the determined molybdenum percent (2,19%) it's situated toward the inferior limit. CoCrMo alloy ranges within ASTM F75 standard from both chemical composition and structural points of view. Microstructure in casting state is made out of a rich cobalt matrix, segregated alpha phase, inter-dendrites, and at the grains separated limit compounds. Ti6Al4V alloy type “E.L.I.” (Extra Low Interstitial) has a structure made out

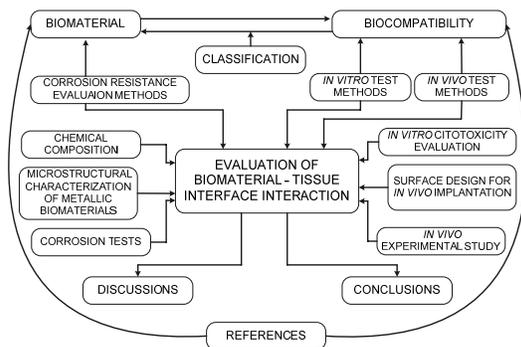


Fig. 1. Schematic representation of the experiments program.

of longed alpha phase grains, light color, inside a transformed beta phase matrix.

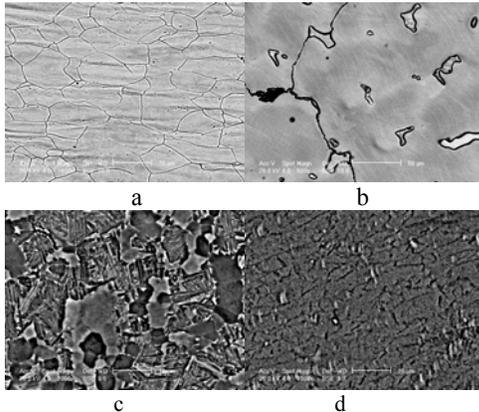


Fig. 2. The aspect of the microstructure of: a) stainless steel alloy; b) CoCrMo alloy; c) Ti6Al4V alloy; d) pure titanium.

Alpha phase, according to ASTM F136 point 8.1, is low dispersed, as well as the beta phase. Alpha phase is light plastic deformed, and the structure resulted from the thermal treatment is a typical one in which the „beta-transus” temperature was considered. The titanium grades 3 and 4 are in plastic deformation state (with a 30% deformation grade) and the grains have diameters between 10 and 100  $\mu\text{m}$ .

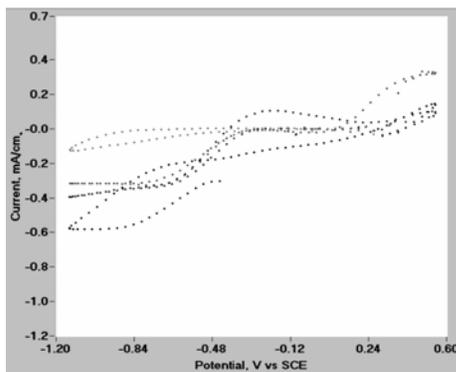


Fig. 3. Voltametric cycles obtained after the metallic biomaterials corrosion testing.

During these tests, we followed the evaluation of general corrosion compartment and localized corrosion in artificial physiological solution. The practical experiments were made on an AMEL 2049 potentiostat and an electrochemical cell with three electrodes, the reference electrode being Calomel SCE, the contraelectrode from platinum and working electrode. The temperature was 37°C, maintained with an ultrasonic bath and Finosonic thermostat. Using Voltassist software, we tested voltametric cycles between 1200 mV and -600 mV, with a scan rate by 1mV/s.

In stainless steel case, the voltametric cycle indicate an active zone until a value of potential of -0.43 V, when a

-0,02  $\text{mA}/\text{cm}^2$  value of current density is achieved, which is corresponding to maximum corrosion current intensity and which is decreases in the next potential interval until the passivation current value equal with 0,017  $\text{mA}/\text{cm}^2$ . This current is mentained until the transpassivation domain. On the return cycle we did observed the existence of a small hysterezis and of a few variations of the current in the passive domain, which show us a sensibility for localized corrosion. In the CoCrMo alloy case, the intensity of the maximum corrosion current appears at a corrosion potential by -0.21 V. After this active area the alloy is passivated, the passivation domain being between -0.210 and 0.17  $\text{mA}/\text{cm}^2$  and -0.66 V, at a passivation current by -0.3  $\text{mA}/\text{cm}^2$ . On the return domain it is possible to observe a small hysteresis and variations in the passivation domain, which show a small sensibility for crevasse corrosion. Pitting and crevasse corrosion, have almost the same mechanism, although initiated by the differential airy phenomenon, but the pitting corrosion it is caused by submicroscopic defects, in special by MnS inclusions (plastic inclusions) orientated on deformation direction, and crevas corrosion which it is obtained because of the macroscopic defects of the surface oxide layer.

This kind of defects could be generated by the roughness of the surface; respectively because of the presence of the unhomogeneities which is bringing on surface some cells with differential airy phenomenon.

In the Ti5Al4V alloy case the active domain it is very small, the current density growing from -0.15 to 0  $\text{mA}/\text{cm}^2$ , which is maintained on a potential domain from -0.96 to -0.57 V. The return sector show a very small hysteresis, smaller then other alloys and without significant variations of the current on this sector. From this voltametric cycle result a very good demeanor at general and located corrosion, determinate by appreciation of the maximum value of the corrosion current, of the passivation current and of the potential area, when the density of the passivation current it is maintained constant. The commercial pure titanium show an anodic curve with an active domain reaching the current density by 0.19  $\text{mA}/\text{cm}^2$ , at a -0.61 V potential value. The return area show a very small hysteresis, which means that this biomaterial it is very sensible at localized corrosion forms. From the analysis of the quantitative and qualitative data, the studied biomaterials present a good corrosion resistance, but the premises related to the cell and tissue adhesion to their surfaces in the micro flaws caused by the grain limits and inclusion state are not conclusive yet. In the topical context, starting from the results and accumulated experience, we aimed to apply the erythrocyte viability estimation criteria on detecting and surveying the induced effects of metallic biomaterials. To confirm the results, we made a cytotoxicity test of metallic materials using a fibroblast cell line obtained from monkey embryo. Extending erythrocytes incubation time with the different metallic samples up to 96 hours, conceded to a clear hierarchy of their toxicity, according

to the rest of viable cells percent and of apoptosis erythrocytes.

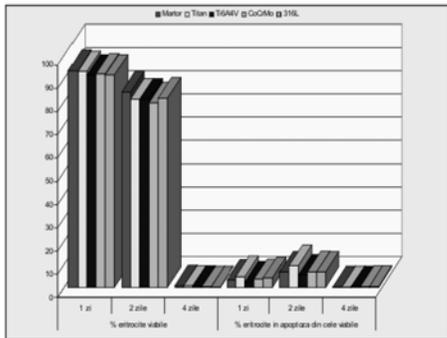


Fig. 4. Variation of the viable erythrocyte percents, compared with apoptosis erythrocyte from the still viable ones, as a function of incubation time.

The most toxic one, proves to be the 316L austenitic stainless steel alloy type sample, and the less toxic one is the pure titanium sample, having 69% of viable cells compared to the 75.5% of witness sample. After 24<sup>th</sup> hours of incubation in the presence of the metallic samples it is easy to observe that we don't have a toxic effect or cellular lyses, or induction of significant apoptosis compared with witness sample.

The results comparison obtained from flow biometry after 48<sup>th</sup> hours confirm the more toxic effect of the stainless steel sample. Time prolongation at 96 hours of erythrocyte incubation permitted a hierarchical appreciation of samples toxicity. From the Fig. 5 where we expressed in increasing order the samples toxicity, result very clear the biomaterials ordering from the *in vitro* compartment point of view. Hereby, the most toxic it was the austenitic stainless steel, succeeded by Corm alloy, Ti6Al4V alloy, respectively commercial pure titanium. This ordering it is a little bit different than the established order resulted from the corrosion tests, because of the exchanging of the positions occupied by Corm alloy and austenitic stainless steel. This inversion of the positions due of the chemical composition, Corm alloy has a smaller concentration of cytotoxic potential elements like nickel which can disperse into the growing cellular medium. The next step was the implantation. The samples were prepared with equal dimensions and surfaces and implanted in the tibiae of the same organism (rabbit). At time intervals of one, three and six months, we obtained the biological samples by subject sacrifice. This sample was imaginatively investigated with an electronic microscope ESEM type. Pursuant to standards for biocompatibility, in the case of animal tests, for the evaluation of the biomaterial – hard tissue interaction, four samples were implanted into the rat tibiae. With the samples which contain the metallic implants, it were extracted, from the same level pure biological samples (witness samples) for a comparison analysis and a better interpretation of the experimental results. These samples were investigated by scanning electron microscopy, using

a 5 Torr water vapor pressure in the chamber of the sample. The bone samples containing metallic implants were analyzed at the surface level and intramedular. At the surface analysis we observed the morphologic aspect of the bone tissue.

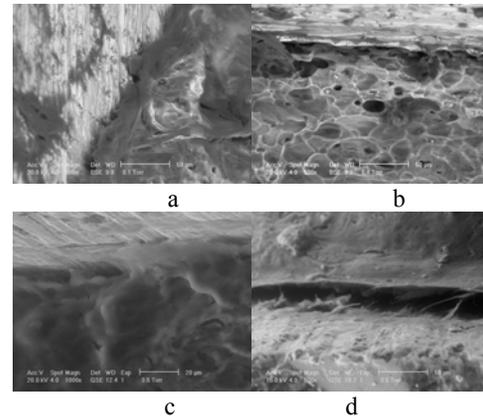


Fig. 6. ESEM micrographs obtained after one month of implantation in rabbits: a) titanium; b) Ti6Al4V alloy; c) CoCrMo alloy; d) stainless steel alloy.

The observed elements described by ESEM images demonstrate the existence of a complex process for healing around implants. After one month from implantation, the peri-implantar tissue present a conjunctive fibrous, rich cellular populated, which suffer intense processes of bone transformation. After three months from surgical procedure, the fibrous peri-implantar tissue almost perished and the new bone begun the functional reshaping process, since after six months from implantation the new sweep bone functional reshaped it is hard to view with the electron microscope at the limits of the initial bone defect.

Depending implant type, the transformation succeeded with few individual characteristics. The morphology of the new peri implantar developed bone around stainless steel implant confirms the presence of numerous new blood vessels with intense calcification. The fibrous metaplasty of the peripheral granular tissue it's totally substituted by the bone metaplasty process. Albeit compared with the morphological characteristics observed for the titanium implant, the reshaping processes are delayed; we could not observe intolerance biological reactions at this kind of biomaterial. The new bone format around the Ti6Al4V alloy implant show new blood vessels in an intense functional reshaping process by creation of a reorganization of a intern tibial bone wall. In comparison with the stainless steel alloy, the morphological image of the intramedular bone tissue new format around the CoCrMo alloy implant show the fact that this implant presents better characteristics for the tissue organization and reorganization during biointegration. The morphological characteristics of the new bone tissue developed on the pure titanium implant surface show a stage of mature functional reorganization, by complete disappearance of vascular drawing calcificated intra

medulary. In the surface implants complexity context, because of the different structures, roughness and chemical compositions of them, the aim of this paper was to analyze the processes from the biomaterial – tissue interface. For reaching this objective, we made in this order, determinations for micro structure of the biomaterials, corrosion resistance, the comportment and the influence of the cellular cells and in the final *in vivo* tests on animals.

We discussed the metallic biomaterial implants interactions with different structures and roughness of the surfaces with biological tissues, which included discussions about the mechanism of the implant incorporation and about the development and modification of the tissues during reorganization after implant insertion. Different grades of the surface preparation was measured and compared with the surface of the bone, because it's known that these surfaces were covered in many cases with bone deposits and from that reason they are serving like fixation points for the new grown bone. The structures of the surfaces were measured, characterized and correlated with the morphometrical evaluations of the tissue – biomaterial contact. From these observations and by correlation with the specialty literature, we extract conclusions for the mode and the possibilities for metallic implant surface topography optimization, with medical applications. The validation of the obtained results was possible by the imagistic observations of the surrounding tissues, unaffected during scanning electron microscopy evaluations.

This study made possible the understanding of the inner cellular mechanisms and the interaction between metallic implant – biological tissue. Due to the design and use of an absolutely new form for the metallic implants used to make the *in vivo* research, the study effectively contributes to the reduction of the number of testing animals needed for further research.

#### 4. Conclusions

Regardless the alloy type, at one month, three months and respectively six months time intervals we noticed the bio integration happening. For none of the alloy type, periimplantar bone absorption processes weren't observed, fact that proves the existence of a favorable interaction between the biomaterial and the biologic structure. Analyzing from the morphological point of view we observed different perimplantar bone tissue reshaping stages. In the case of the Ti and Ti6Al4V implants, the reshaping processes are found in a very advanced state, moderate for the CoCrMo alloy and incipient for the 316L stainless steel implant. As a function of the compositional type of the implant and its surface preparation level we can conclude that:

- Regardless the alloy type there is a direct proportionality between the implant surface roughness and the peri-implantar tissue speed of transformation into mature bone tissue (inflammatory

tissue with neo formation vascular plexus, fibrous tissue, new formed bone tissue)

- The maximum adhesion of the bone tissue appears at P320 roughness (the biggest) of the Ti implant, the hiatus being minimum.

The metallic implants surfaces can be characterized exactly by the standard procedures regarding the height, wideness and surface roughness, along with the ones regarding the chemical composition, because of the presence of impurities function of the method of obtaining of the implant. The surface structure must be irregular and must allow the mechanical fixation between the surface irregularities and bone. As a function of the surface roughness and its structure, the biologic tissue quantity from the implant surface grows with the increase of the surface roughness. The implants structured surfaces, which permit the mechanical fixation, ensure a better and much rapid incorporation of the implants into the hard tissues, fact demonstrated by observing the perpendicularly inserted bone cells, the speed of reshaping being low at the interface. A smaller contact of the bone with the interface is needed in order to ensure a better fixation of the implant and the possibility to obtain an increased elasticity of the obtained couples.

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