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In Vitro study for new Ti-Mo-Zr-Ta alloys for medical use

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Abstract. In recent years, researchers have shown a particular interest in improving titanium alloys in the field of medicine. Paper presents a vitro study for three alloys of Ti-Mo-Zr-Ta. This alloys was obtained in order to use them in medical applications as well as in paper studies present a lower level of cytotoxicity with future tissues.

1. Introduction

Biomaterials play an important role in many medical applications, classified in four major classes (metallic, ceramic, polymers, composites). They are find in different applications in domains like: urology, dentistry, orthopedics, cardiology, etc. [1-4].

Ti-based alloys are metallic biomaterials, usually vey used for superior properties beside classical alloys like Co-alloys and stainless steel. Compared to stainless steels and Co-based alloys, titanium alloys have a better biocompatibility. Stainless steels and CoCr alloys are prone to corrosion, releasing metal ions into the body that can cause side effects.

For the functionality of a material for use in medical applications, three important factors are taken into account: biocompatibility, mechanical properties and corrosion resistance [4-6].

The biocompatibility of a material can be defined in the sense that it produces desired or tolerated reactions in a living organism. The metals, in contact with the biological body, give complex effects, producing a series of biological reactions depending on the concentration, the exposure time, etc. According to the biological interaction method, the metals are divided into: *metallic elements* required in very small concentrations for the living organism called essential elements, including cobalt, manganese, zinc, magnesium, sodium, potassium etc.; *elements that produce toxic effects* for the body if present at higher concentrations, the cytotoxic effect being demonstrated by the cell culture system, such as arsenic, cobalt, nickel etc .; *metals with allergic potential* (nickel, cobalt and chrome) are considered to be highly allergic to the body.

The overall strategy for testing biomaterials involves assessing them in two ways [7, 8]: (i) "in vitro" assessment performed on cell cultures or in the blood; (ii) "in vivo" assessment in animals.

Depending on the material-body interactions and the possible reactions occurring, the biocompatibility of the material can be assessed.



When selecting a particular implant material, account must also be taken of the health of the implantation area, both in a healthy and uninfected context, and in a morphologically relevant tissue quality of implantation. The healing phase of the living organism after surgical intervention takes into account the long-term resistance of the implant [9, 10].

Regardless of the medical application, making a biomaterial depends on several requirements: it is not toxic, it does not contain filter products, it does not cause allergic, carcinogenic, teratogenic effects (generated by morphological abnormalities), it does not cause rejection phenomena body, do not alter the blood composition and disrupt the coagulation mechanism, not modify the biological pH, do not contain hydrophobic or hydrophobic sites that promote cellular penetration and adhesion [11, 12].

Current paper present cytotoxicity "in vitro" assessment of three alloys of TiMoZrTa (TMZT). This is necessary to determine the processes taking place at the material interface - biological environment.

2. Experimental procedure

Three Ti-based alloys were obtained in vacuum arc remelting MRF ABJ 900. As raw materials was used elements with a high purity: Ti - 99.8%, Mo - 99.7%, Zr -99.2% and Ta - 99.5%. In the process of obtaining, alloys were remelted at least seven times, for homogeneity. In Table 1 is the chemical composition of the alloys obtained.

Table 1. Chemical composition of the TiMoZrTa (TMZT) sample after melting.

Element	Mo	Zr	Ta	Ti	Named in paper
wt.(%)	13.56	6.34	4.64	balance	Ti15Mo7Zr5Ta
	12.33	6.80	7.24	balance	Ti15Mo7Zr10Ta
	12.56	6.99	14.45	balance	Ti15Mo7Zr15Ta

Cell viability for TMZT alloys was tested by the MTT test (Tetrazolium Salt Method) [13-15] using a tetrazolium salt, wich in in aqueous solution at neutral pH, has a yellow color and is capable of penetrating the cells.

To evaluate cytotoxicity, 3 metal samples of TMZT alloys was cut to size 5 mm x 5 mm x 3 mm, then prepared specifically for the MTT assay and placed into well plates together with the culture medium. Cell viability was expressed as a percentage by reference to control well plates (wells containing complete culture medium).

Samples of specified sizes were prepared by exposing them for 30 minutes on each side to the UV action in a transiluminator. After, they were plated in 24-well culture plates for seeding cells in order to study cell viability. The prepared samples were kept under sterile conditions in an incubator at a temperature of 37 ° C, in a 5% CO₂ atmosphere and a high relative humidity (> 95%).

3. Results and discussions

A method successfully used for assessing the biocompatibility of metal alloys is that based on the effects of cell cultures, this method becoming the primary way of preliminary testing of in vivo biocompatibility studies for the analyzed biomaterials.

Preparation of cell cultures consisted in selection of HOS-human osteosarcoma cells (CLS, Eppelheim, Germany) having an osteoblastic phenotype. The cells were thawed and suspended in 20 ml of MEM culture medium (Dulbecco's modified Eagle's medium) supplemented with 10% FBS, 2% L-glutamine and 1% antibiotic (penicillin-streptomycin) in flasks culture (parallelepipedic plastic recipient with a surface area of 75 cm²). Affinity was reached at 72 hours, after which the culture medium was removed, the adherent cells were washed with PBS (Phosphate Buffered Saline Solution), then cleaved with an EDTA trypsin solution centrifuged and resuspended in 1 ml complete culture medium and subsequently counted with a Neubauer counting chamber. From the 1 ml (medium + cell) work suspension, 4 x 75 cm² flasks were seeded and kept in a humidified incubator at 37°C and 5%CO₂. After 3 days the confluence in these flasks was complete; the cells were detached

by trypsinization, washed with PBS, centrifuged, resuspended in 1 ml of complete medium and counted for seeding on 24-well cell culture plates.

The study of the viability of HOS cells co-incubated with samples belonging to TMZT alloys consisted of assessing cell viability at 3 and 9 days respectively by seeding the cells after passage on the metal samples in 24-well plates of 1×10^5 cells / well / 1ml MEM complete (MEM with 10% FBS and 1% antibiotic-antimycotic), incubated in humid atmosphere at 37 ° C and 5% CO₂. For conclusive results, cells were seeded in wells containing only complete culture medium (referred to as Control wells), thus comparing with the results of samples from TMZT alloys. For the 3-day test, after 24 hours the medium was removed by aspiration; washing with PBS and adding completely fresh medium was performed. For the 9-day test, the culture medium supplemented with the extract was first changed after 24 hours and then at 48-hour intervals. For this, the medium was removed by aspiration; washing with PBS and adding fresh medium completely.

Values from the HOS cell viability study co-incubated with metal samples from TMZT alloys are shown in Table 2.

Table 2. Cellular viability assay results of HOS cells co-incubated with metallic TMZT alloys.

Alloy	Viability – 3 days (%)	Viability – 9 days(%)
Control	100.00	100.00
Ti15Mo7Zr5Ta	72.55	66.55
Ti15Mo7Zr10Ta	78.09	75.32
Ti15Mo7Zr15Ta	67.71	61.02

The values recorded for TMZT alloys for the three and nine day values, shown in Table 2 were expressed as percentages from control wells. Cell viability for three days co-incubation of HOS cells with TMZT alloys ranged from 67.71% (Ti15Mo7Zr15Ta) to 72.55% (Ti15Mo7Zr5Ta) compared to control wells. Cell viability recorded for nine days of co-incubation of TMZT alloys ranged from 61.02% (Ti15Mo7Zr15Ta) to 75.32% (Ti15Mo7Zr10Ta) relative to the control wells.

In Figure 1 and Figure 2, the cell viability result for TMZT alloys are presented graphically at three respectively nine days co-incubation.

The obtained results reveal a low level cell viability after 3 days for Ti15Mo7Zr15Ta and a level higher for the Ti15Mo7Zr10T alloy.

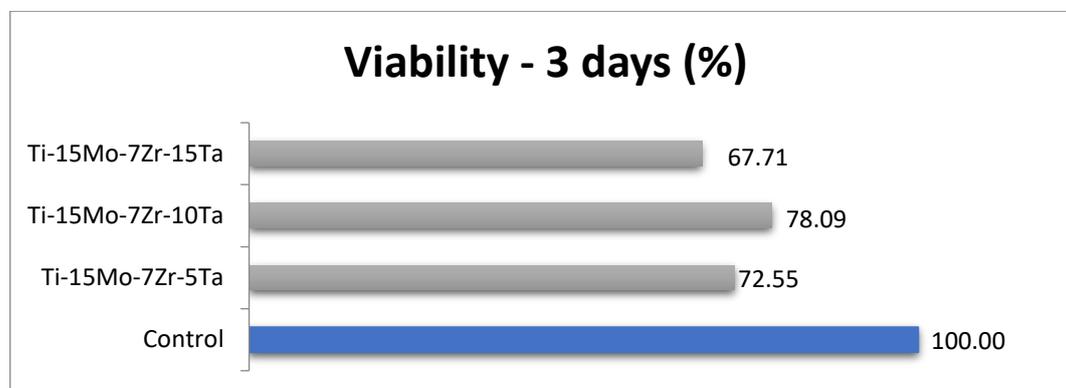


Figure 1. Results of the MTT test for the cell viability study of HOS cells co-incubated with TMZT alloy samples for 3 days of co-incubation.

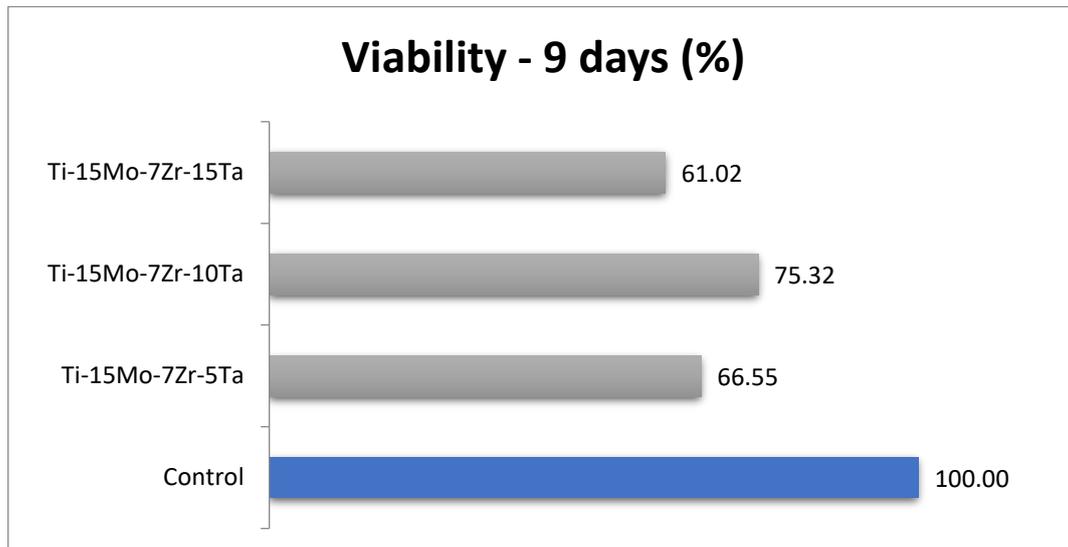


Figure 2. Results of the MTT test for the cell viability study of HOS cells co-incubated with TMZT alloy samples for 9 days of coincubation.

The results obtained after 9 days recorded in the TMZT alloys a slightly lower viability level for the Ti15Mo7Zr15Ta alloy. However, these differences are not very significant and could be attributed to the compositional characteristics of the samples taken into study (release of cytotoxic metallic or surface energy) or to the differences between the topographical characteristics of the samples as the samples had different finishing degrees and randomly, which significantly influences cell anchor and proliferation in vitro.

Researches in the field carried out on pure metals (Ag, Al, Cr, Cu, Mn, Mo, Nb, V, Zr) by cell viability tests and compared to pure titanium made a classification of metal toxicity as follows: Ti > Mo > Nb > Zr > Cr > Mn > V > Ag > Al > Cu. Numerous studies indicate that titanium alloys release lower amounts of metal ions in the body than those containing aluminum and vanadium [1-7].

In the case of TMZT alloys analyzed, their low cytotoxicity was due to the presence of alloying elements (Mo, Zr and Ta), which contribute to the formation of stable oxides.

Based on the results, we can say that TMZT alloys have adequate cell viability and can be considered potential candidates for medical applications.

4. Conclusions

When developing an alloy for medical applications, the main features such as biocompatibility have to be considered. In paper three Ti-Mo-Zr-Ta alloys have been studied from this point of view.

Cellular viability assessment studies of HOS cells co-cultivated with metallic samples from TMZT alloys have highlighted that Ti15Mo7Zr10Ta and the rest are non-toxic for both of the time intervals studied, this alloys fit into a low cytotoxicity range. However, the level of viability can not be fully attributed to the toxic nature of the alloys, and complementary studies complementing aspects of alloy-cell interaction such as zeta potential, microgeometric and topographical topography (results from cutting or corrosion) are needed.

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