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Biocompatibility Evaluation of New TiMoSi Alloys

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The mechanical properties of materials used for orthopaedic implants are of major importance for the bone remodeling processes and for the bio-functionality. Young's modulus for metallic biomaterials is higher than for cortical bone (7–35 GPa): stainless steels – higher than 208 GPa, titanium and usual titanium alloys — higher than 102 GPa, Co-Cr alloys — above 201 GPa. As a result, the mechanical mismatch implant — tissue generates bone atrophy due to the "stress shielding" effect. The paper contains elaboration and characterization of a new two Ti-based alloys for medical applications. Samples were cuted and tested to obtain the main mechanical properties (hardness, indentation etc.), after that were measured the cell viability on new Ti alloys. The results obtained promise new alloys with improved properties compared to the classical alloys.

topics: biomaterials, Ti–Mo–Si alloys, low elasticity modulus, XRD patterns, cell proliferation

1. Introduction

Metallic biomaterials are widely used class of materials for making implants, prostheses and medical instruments, mainly because they have very good mechanical properties, are resistant to corrosion, and have acceptable biocompatibility [1–4]. Titanium and titanium alloys are the most commonly used metal materials for implants. The best known titanium alloy is Ti_6Al_4V, which has remarkable physical, mechanical, chemical and biocompatibility characteristics. However, as Vanadium is toxic, it began to be replaced with different biocompatible elements, i.e., Fe, Si, Ta, Mo, and Zr [5, 6].

Biocompatibility is a complex concept that takes into account all the processes that take place in the interaction between biomaterial and a living organism. Physical-mechanical, chemical, biological and surface-specific processes play a determinant role in the selection of an alloy for medical applications [7–10].

The advantages of using titanium in implantology consisted of resistance, reliable technological process of obtaining, easy processing by conventional and unconventional means, acceptable price. An essential aspect is represented by the fact that it is covered with a thin layer of oxide that prevents corrosion and allows osteointegration (passivation phenomenon), gives it good compatibility, being well tolerated by the soft and bone tissues [11–13].

Since alpha beta Ti-based alloys (Ti_6Al_4V, C.P. Ti) remain the main titanium materials used for current medical applications, the latest research has shown a great increase in the synthesis of the metastable beta titanium alloys designed specifically for biomedical applications. To remove the toxic elements currently used as titanium alloying elements, we have developed a new titanium system (TiMoSi) with good properties. Originally system meant to address the dual requirement of low Young's modulus, approaching that of the bone, and enhanced biocompatibility. The present paper presents a structural, mechanical characterization and verification of the biocompatibility of the elaborated alloys.

2. Experimental Procedure

In order to obtain the new system purpose, it has been chosen to use a vacuum arc reflowing plant MRF ABJ 900 and were used raw materials with a high purity: Ti — 99.80%, Mo — 99.70%, Si — 99.20%. Two alloys were produced $Ti_{15}Mo_{0.5}Si$ and $Ti_{20}Mo_{0.5}Si$. The chemical characterisation of the alloys was done with EDX, as an average on multiple points for a precise determination. The EDX is coupled to a SEM VEGA II LSH manufactured by the TESCAN Co., Brno, the Czech Republic. The chemical composition with a uniform distribution of elements and a homogeneous alloy is shown in Table I. The structure analysis was performed using the ZEISS AXIO Imager A1m optical microscope.

TABLE I

Chemical compositions of elaborated TiMoSi alloys.

Alloy	Titanium [% mass]	Molybdenum [% mass]	Silicon [% mass]
$Ti_{15}Mo_{0.5}Si$	83.79	15.93	0.28
$Ti_{20}Mo_{0.5}Si$	80.29	19.44	0.27

The surface structure of the samples was analyzed by a TF-XRD: model RNT-2500, Rigaku Co., Tokyo, Japan. The indentation tests were performed using CETR UMT-2Universal MicroTribometer. MC3T3-E1 cells were seeded on cp-Ti, $Ti_{15}Mo_{0.5}Si$, and $Ti_{20}Mo_{0.5}Si$ specimens in 24-well culture plate at density 2×10^4 cells/well and cultured at 37 °C, 5% CO_2 in α-MEM (Gibco, USA) supplemented with 10% (v/v) fetal bovine serum, 1% (v/v) penicillin and streptomycin. After 3 and 7 days, cell count reagent SF (Nacalai tesque, Kyoto, Japan) was added to each well and incubate for 2 h in the incubator. Then, 100 μl of the medium was transferred to a 96-well plate and the formazan product was quantified by measuring the absorbance at 450 nm using Microplate reader (iMark^{TM}, Bio-rad, USA). In this experiment, three specimens were prepared for each type of samples. The alloy samples were immersed in 2 mL of phosphate buffered saline with gently shaken at a speed of 50 strokes/min at 36.5 °C. After predetermined periods up to 14 days, the Sr^{2+} and Ag^{+} ion concentrations in the FBS were determined by inductively coupled plasma emission spectroscopy (ICP, SPS3100, Seiko Instruments Inc., Chiba, Japan) [7].

3. Results and discussions

Table I presents the mass percentages of the elements identified in the composition of the alloys, the percentages of the elements varying with small differences from the theoretical load calculation. The analysis on the chemical composition obtained, have revealed that the main elements identified in the elaborated alloys are: Ti, Mo, Si, without the existence of inclusions inside the alloys.

In order to investigate the metallographic structure, the preparation of the metallographic samples of the TiMoSi experimental alloys comprised a succession of stages: cutting to corresponding dimensions (e.g. 10 mm×10 mm×5 mm), embedding in epoxy resin, sanding and polishing at specific speeds, attack chemically with reagent (10 ml HF, 5 ml HNO_3, 85 ml H_2O, for 30 s). After completing the steps, the samples were analyzed under the optical microscope at different magnification powers to obtain detailed images on the microstructure. Figures 1, 2 show the images obtained by optical microscopy for TiMoSi alloys.

In order to evaluate the hardness of the TiMoSi alloys, A Wilson Wolpert universal hardness tester 751 N model, by Wilson Instruments an Instron

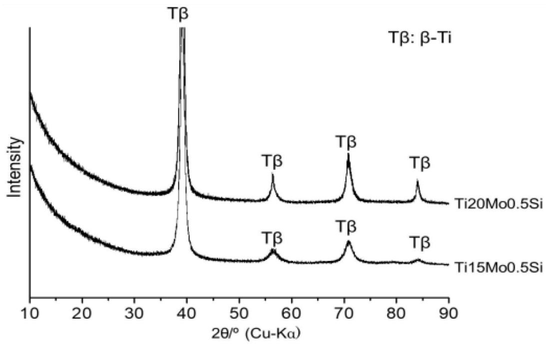

Fig. 1. Optical microstructure of Ti_{15}Mo0.5Si at (a) 100× and (b) 200×, and respectively, Ti_{20}Mo0.5Si at (c) 50× and (d) 100×.

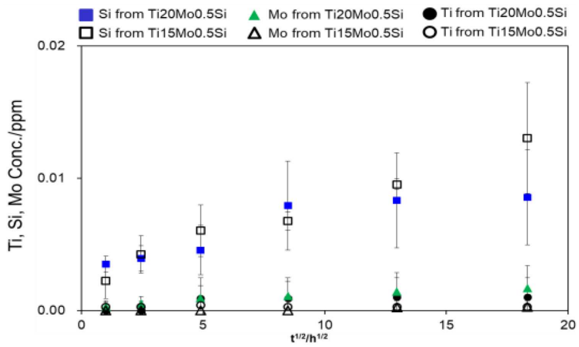

Fig. 2. XRD patterns of TiMoSi alloys.

Company, Heerlen, The Netherlands, was used for the Vickers hardness measurements, with a load of 9807 N during 12 s.

Figure 3 shows the structure of TiMoSi alloys with aspects of the specific grains of titanium alloys. The experimental tests consisted of five determinations in different areas on the surface of each sample. The analysis of the alloy hardness aspects was done by the Vickers method and the samples were tested at a 50 gf (HV 50).

The hardness values were 361 for $Ti_{15}Mo_{0.5}Si$ and 165 for $Ti_{20}Mo_{0.5}Si$. Three determinations for each alloy were performed to determine the values as accurately as possible. After the evaluation, using UMT 2 software, the indentation curves (depth vs. force) of TiMoSi alloys were plotted using the VIEWER program.

The samples were prepared with dimensions of 17 mm×5 mm×5 mm and the surface was metalographically prepared until the metallic gloss was obtained. The testing was done under dry conditions. A Rockwell diamond-type penetrator was used having incisor cone an angle at the 120° applying a force of 5 N. The resulted values are 19.82 for $Ti_{15}Mo_{0.5}Si$ and 37.53 for $Ti_{20}Mo_{0.5}Si$

The mechanical properties of the biomaterials are dependent on the chemical composition, structure but also on the function that the implant has to fulfill in the living organism and to have

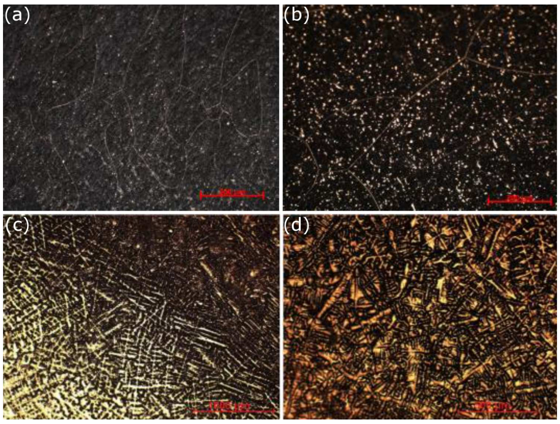

Fig. 3. Concentration of Ti, Mo and Si ions released from TiMoSi alloys as a function of square root of soaking time in PBS, which was measured by ICP.

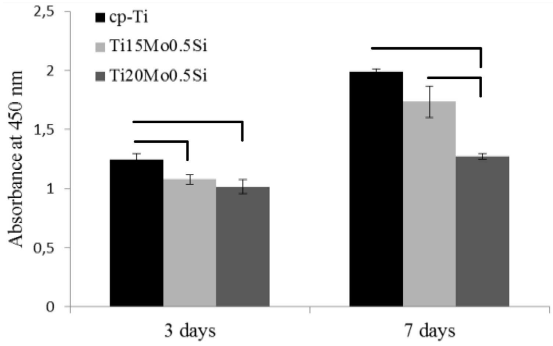

Fig. 4. Proliferation of MC3T3-E1 cells on TiMoSi alloys after 3 and 7 days of culture. Statistically significant digits of reported P values are less than 0.05, 0.01, 0.001, according to the error bars..

properly similar properties to the tissue they replace. The modulus of elasticity is a very important criterion that underlies the choice of metallic materials used in orthopedic applications, and must be as close as possible to that of the human bone (17–30 GPa). According to the literature, CoCrMo alloys (210–253 GPa) and stainless steels (190–210 GPa), presents very high values of Young modulus beside the investigated TiMoSi alloys and the values closer to those of human bone. TiMoSi modulus of elasticity results, reveal significant improvement of the mechanical characteristics compared to titanium alloys: $Ti_6A_{l4}V$ (100–114 GPa) and C.P. Ti (102–104 GPa).

XRD patterns confirmed that both the specimens are consisted of single phase of beta titanium alloys, as shown in Fig. 1.

Figure 2 shows release of Ti, Mo and Si ions into PBS, examined by ICP. Release amount of these ions were as low as less than 0.02 ppm even after 14 days soaking, indicating that both alloys have good corrosion resistance. It is noted that slightly higher amount of Mo ions from $Ti_{20}Mo_{0.5}Si$ was detected compared to that from $Ti_{15}Mo_{0.5}Si$.

Proliferation of MC3T3-E1 cells on these alloy specimens was examined. As shown in Fig. 4, both the alloy specimens exhibited slightly lower absorbance values compared to cp-Ti after 3 days: 86% for $Ti_{15}Mo_{0.5}Si$ and 81% for $Ti_{20}Mo_{0.5}Si$. The absorbance values increased for cp-Ti and $Ti_{15}Mo_{0.5}Si$ at same degree, while it was kept for $Ti_{20}Mo_{0.5}Si$, indicating that cell proliferation was suppressed on $Ti_{20}Mo_{0.5}Si$.

These results suggest that $Ti_{15}Mo_{0.5}Si$ has a surface preferred to cell proliferation compared to $Ti_{20}Mo_{0.5}Si$. Correa et al. reported no cytotoxicity against MC3T3-E1 on Ti-15Zr-Mo regardless of the content of Mo from 0 to 20% [14]. Recently, Terpiłowska et al. reported dose-dependent cytotoxicity of Molybdenum ions on BALB/3T3 and HepG2 cells [15]. In the present study, the suppression of MC3T3-E1 cell growth might be explained by larger release amount of Mo ions from $Ti_{20}Mo_{0.5}Si$. Further study is required to clarify the effect of Mo content in the system of TiMoSi alloy.

4. Conclusions

Regarding the results obtained of TiMoSi alloys compared to the other biomaterials, these alloys have the closest value to that of the human bone, which recommends their use in medical applications. Both the Ti_{15}Mo0.5Si and $Ti_{20}Mo_{0.5}Si$ have good corrosion resistance while the former alloy exhibited better cell proliferation.

Acknowledgments

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