”CAROL DAVILA” UNIVERSITY OF MEDICINE AND PHARMACY BUCHAREST
PhD DEPARTMENT
DENTAL MEDICINE

PhD THESIS

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2024

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Ti based bioalloy for obtaining metal components for implant supported restorations

PhD THESIS ABSTRACT

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Introduction

The use of titanium as biomaterial is based on its properties such as: high specific resistance and corrosion resistance. Also, commercially pure titanium when placed in contact with human tissues does not trig allergic reactions, being considered the most biocompatible metallic biomaterial.

At this moment, for manufacturing dental implants and connectors between implant and prosthetic restorations are used mostly commercially pure form of titanium or titanium alloys that contains Al and V, more precisely Ti-6Al-4V. In the composition of titanium alloys, Aluminum is a stabilizing element for α phase, while V is a stabilizing element for β phase. Both elements, aluminum and vanadium, are known as toxic and allergic elements for human body. Numerous studies have shown that titanium alloys containing Al and V, can slowly release ions of aluminum and especially vanadium that can induce adverse reaction in surrounding tissues or even unwanted immunologic response [1] [2].

Other studies have shown that Al and V can be replaced by other chemical elements with a lower or none toxic potential. Thus, elements such Fe, Mn, Ta, Nb, Mo or Zr can be used in Ti base alloys or in High Entropy alloys, with similar properties with Ti alloys that are current in use[3] [4].

Starting from elements afore mentioned, the purpose of this study is to evaluate the way that chemical elements considered inert or high biocompatible can be integrated in chemical formulas of Ti base alloys, to partially or totally replace chemical elements that can generate local or general side effects as Al and V. In the same time, the effect on physical and chemical properties induced by changes of alloys composition should be evaluated.

Working hypothesis and general objectives

The studies carried out in this PhD Thesis had as their starting point the composition of the most common alloys used to make dental implants and prosthetic restorations made of Ti-based alloys. Currently, the most widely used alloys in medicine and also in dentistry are the commercially pure forms of Ti and the alloy formula Ti-6Al-4V. Although the use of these alloys is based on years of research and clinical tests, we must not neglect the studies that have shown the harmful effect of Al [5] [6] [7] and V [8] [7] when they are released from the Ti-6Al-4V alloy structure. Studies carried out by Perl 1985 [5], Domingo 2002 [8], Klotz et al 2017 [6] or Jaishankar et al 2014 [7], showed the harmful effects that Al and V can have on the body, a situation that it can also happen with dental implants. Hence the increased interest, as can be noted in Chapter 2, for finding new alloy formulas that partially or totally replace Al and V in the structure of Ti alloys, with properties at least similar or even superior to the alloy formula Ti-6Al-4V.

The purposes of the conducted studies were related to the possibility of obtaining and testing new alloy formulas containing chemical alloying elements that are not harmful to the human body.

The general objectives of the studies were the following:

- Obtaining new Ti base alloys in laboratory conditions, within the capacity of the melting machines;
- Compositional analysis of the new alloys;
- Physical analysis of the new alloys;
- Biological evaluation;

Chapter 4 - Effects of chemical composition on the microstructural characteristics of Ti-Nb-Ta-Zr alloys

4.1 Purpose of the study

There are numerous chemical elements considered to be non-toxic and non-allergenic, through reported data on cell viability for pure metals, polarization resistance and tissue compatibility, that can be alloyed with Ti, such as Nb, Ta, Zr, Sn, Mo, Fe or Hf. The use of such elements would prevent the situations encountered in the case of Ti alloys with Al and V content. Some of these elements are already used to stabilize the β phase (Fe, Nb, Ta).

Since the field of Ti alloys is still incompletely known, we tried through this study to see the following:

- if it is viable to obtain, under laboratory conditions, some Ti base alloys by combining Ti with Nb, Ta and Zr, in different concentrations of the component elements;
- analysis of the experimental alloys obtained;
- the analysis of the microstructural properties compared to other forms of Ti alloys existing on the market, but also to those of the tissues replaced during surgical and dental treatments:
    - o Microhardness;
    - o Shear resistance;
    - o Microstructure (optical/SEM).
- Veneering with ceramic masses of the experimental alloys and the analysis of the adhesion of the ceramic masses to them.

4.2 Material and method

For this study, three Ti base alloy formulas were proposed by combining Ti in different concentrations with Nb, Zr and/or Ta: Ti1 (Ti, Nb, Zr); Ti2 (Ti, Nb, Ta, Zr) and Ti3 (Ti, Nb, Ta, Zr). To compare the mechanical and microstructural properties, the Ti-9Al-3.6V alloy was used.

The production of the alloy samples as well as their testing was carried out in collaboration with the "Elaboration and Refining of Metallic Materials" Laboratory ERAMET within the Faculty of Materials Science and Engineering, Polytechnic University of Bucharest, laboratory led by Prof. Univ. Dr. Eng. Victor Geanta. The experimental alloys were obtained using the Vacuum Arc Remelting Machine "RAF MRF ABJ 900" of the

ERAMET Laboratory (Fig. 4.1). This device allows obtaining temperatures close to the value of 3500°C, being above the value of the melting point of Tantalum of 3290°C [9].

Fig. 4.1 – The alloying elements placed on the base plate of the electric arc melting machine "RAF MRF ABJ 900"

Since melting using the electric arc involves reaching temperatures of about 3500°C, it is normal that during the alloying process, some of the introduced alloys disappear by evaporation or burning. For this reason, the analysis of the chemical composition of the experimental alloys is carried out after obtaining them. The analysis was carried out using the Spark Optical Emission Spectrometer "SPECTROMAXx M", the data interpretation being carried out with the Ti-01 program but also through EDAX analysis.

The microhardness of the alloys was evaluated using the "Shimadzu HMV 2T" machine, using a force of 1N applied for 10s. In order to obtain the most accurate values, the microhardness was measured in 10 different points for each material sample.

Microstructure analysis was performed by optical microscopy (Olympus GX 51) and scanning electron microscopy (SEM) (Inspect S, equipped with EDAX Z2e analyzer), after the samples were previously treated with a metallographic etching reagent.

For this study, Noritake Super Porcelain Ti-22 (*Kuraray*) ceramic mass was used, specially created for veneering on structures made of titanium.

The strength of the metal-ceramic bond was tested by subjecting the samples to axial shear forces, using a universal tensile/compression testing machine, in static and dynamic mode (type UFP400 Germany).

4.3 Results

4.3. Experimental alloys obtained

Using electric arc melting in a "RAF MRF ABJ 900" furnace, the three experimental Ti base alloys were obtained. The chemical compositions of the three alloys as well as the comparison alloy are shown in Table IV.1.

Table IV.1 – The chemical composition of the studied alloys

	Alloys			
Chemical elements (wt%)	***Ti1***	***Ti2***	***Ti3***	***Ti9Al3.6V***
Ti	82,74	76,79	74,40	87,3
Nb	9,26	14	10	-

Zr	8	4,53	8	-
Ta	-	4,68	7,6	-
Al	-	-	-	9,1
V	-	-	-	3,6

4.3.2 Microhardness analysis

The average microhardness values for the four analyzed alloys are presented in Table IV.2.

Table IV.2 – Microhardness values for experimental Ti base alloys

	Ti1	***Ti2***	***Ti3***	***Ti9Al3,6V***
Average value (HV1)	***360***	***319***	***437***	***354***

The highest hardness obtained after testing was found in the Ti3 alloy for which the amounts of Ta and Zr are the highest for the batches studied. Also, the microhardness value for the Ti1 alloy is slightly higher compared to the alloy taken for comparison (Ti9Al3.6V).

4.3.3 Microstructural analysis of the experimental alloys

The three types of alloys were analyzed using both optical and scanning electron microscopy. Also, on specific areas, measurements were made regarding the local chemical composition using the EDS analyzer of the electron microscope.

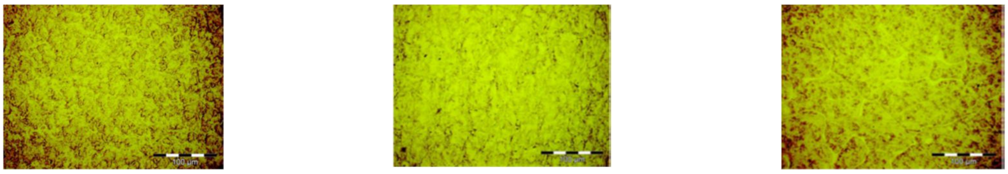

Fig. 4.2 – Optical microscopy analysis of the obtained alloys Ti1 – Ti2 – Ti3 (from left to right**).**

The first experimental Ti1 alloy presents a homogeneous structure with a dendritic aspect, an aspect noticed by optical microscopy (Fig. 4.2). The same dendritic aspect is also found following the electron microscopy analysis (Fig. 4.2).

The addition of Ta as an alloying element in addition to Nb and Zr, the experimental alloy Ti2, does not produce significant structural changes, but generates changes related to the morphological appearance of the alloy, thus globular and lenticular forms are found.

For the third experimental alloy formulation, Ti3, increasing the amounts of Zr and Ta resulted in lenticular separation of the Ti-Zr and Ti-Nb phases with localization in the interdental zones (Fig. 4.3).

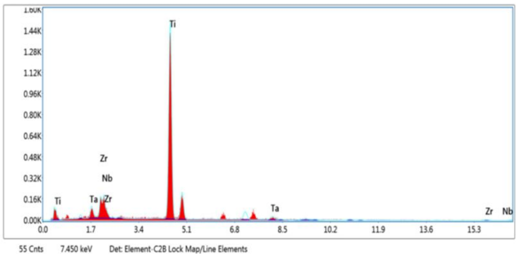

Fig. 4.3 – EDAX analysis of the micro zone analyzed by electron microscopy to establish the positioning of the component elements in the alloy structure.

4.3.4 Veneering of dental ceramic mass and analysis of the interface zone using electron microscopy.

The next stage of analysis involved the mechanical processing of the experimental alloy discs to allow the application of ceramic masses to them, as well as the performance of tests to evaluate the metal-ceramic bond. Thus, from the 3 discs (corresponding to Ti1, Ti2 and Ti3), metal rods corresponding to the test machines were cut (Fig 4.4).

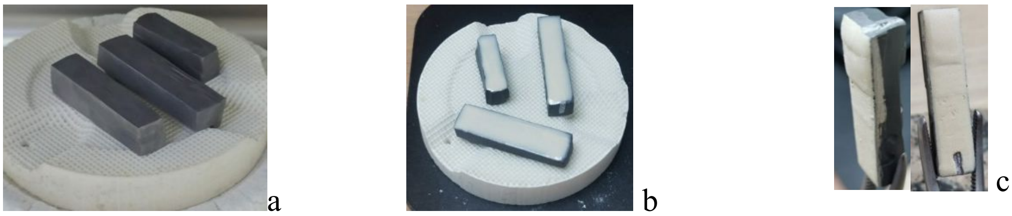

Fig. 4.4 – The appearance of the experimental alloy rods: a) at the end of the oxidation program, b) after applying of the opaque layer, c) after applying of the first ceramic layers

4.3.5 Analysis of metal-ceramic bond, shear strength

After applying all layers of ceramic mass, a part of the applied ceramic was removed to allow the metal-ceramic bond test experiment to be carried out. Part of the ceramic mass was preserved, making a step between the alloy and the ceramic mass (Fig. 4.5).

Fig. 4.5 – The alloy samples on which the ceramic mass was applied, prepared for testing the metal-ceramic bond

The testing of the metal-ceramic bond was carried out using a universal test device in static and dynamic mode of Walter Bay standardized mechanical tests.

The alloy piece was positioned and fixed in the device's clamping system, the test rod was placed in the step made in the ceramic mass, and subsequently a progressive force was applied to the rod in the axial direction until the moment when the metal-ceramic bond yielded (Fig. 4.6).

Fig. 4.6 – Appearance of the tested samples after failure of the metal-ceramic bond.

For each test performed, the test device generated a diagram showing the applied force and the value at which the detachment of the ceramic mass from the surface of the experimental alloys was achieved.

Table IV.3 shows the area of the tested surfaces (the surface on which the ceramic mass was at the time of testing), the value of the force at which the metal-ceramic bond failed, as well as the shear resistance.

Table IV.3 – Shear Strength values for the three experimental alloys

Type of alloy	***Sample surface area (mm^2)***	***Force (N)***	***Shear strength (MPa)***
Ti1	***7,43***	***199,20***	***26,79***
Ti2	***9,26***	***140***	***15,21***
Ti3	***12,91***	***398***	***30,82***

The SEM analysis was not limited to the evaluation of the microscopic appearance, using the EDAX Z2e analyzer, it was also attempted to identify the chemical elements in different points of the surface included in the field of analysis of the microscope.

The values recorded for each analyzed area are presented in Table V.4.

Table IV.4 – The elements registered in the ceramic area, the values are expressed in weight percentages

	C-K	***O-K***	***F-K***	***Na-K***	***Al-K***	***Si-K***	***P-K***	***K-K***	***Ti-K***	***Sn-L***	***Ba-L***
pr1(6)_pt2	3.21	55.89	0.72	7.28	3.53	21.63	.24	3.63	3.88		
pr1(6)_pt3	0.78	47.08		5.44	2.48	23.29		3.11		16.71	1.12
pr1(6)_pt4	0.77	52.51		6.15	3.24	32.02		4.42			0.88
pr1(6)_pt5	0.76	48.57		5.61	2.83	27.46		4.04		9.78	0.94
pr1(6)_pt6	0.94	46.85		4.99	2.48	23.82		3.17		16.46	1.28

As can be seen from the EDAX analysis, the chemical elements recorded in the last 4 analyzed areas are specific to ceramic masses.

4.4 Conclusions

Following this study, the following conclusions can be drawn:

- Experimental Ti based alloys can be obtained in laboratory conditions by alloying with Nb, Zr and Ta;
- Due to the method of obtaining the composition is not homogeneous, there are areas of concentration of alloying elements;
- Changing the composition (adding or not Ta) as well as changing the proportions in which the allied elements are found lead to changing the values of microhardness and shear strength;
- Reducing the amount of Zr leads to a decrease in microhardness and shear resistance, the alloy thus obtained being more malleable (compared to Ti1 and Ti3) when forces are applied.

Chapter 5 - The effect of Fe and Mn on the microstructure and microhardness of titanium base alloys

5.1 Purpose of the study

The purpose of the study is to obtain and analyze some experimental alloys based on Ti in combination with Al, V, Fe and Mn.

5.2 Material and method

This study assumed in the first stage the obtaining of three experimental alloys based on Ti by alloying with the following chemical elements: Al, V, Fe and Mn. To make the alloys, the Vacuum Arc Remelting "RAF MRF ABJ 900" machine of the ERAMET Laboratory, within the Faculty of Materials Science and Engineering, Bucharest Polytechnic University, laboratory led by Prof. Univ. Dr. Eng. Victor Geanta.

The chemical composition of the alloys was determined using the Spark Optical Emission Spectrometer "SPECTROMAXx M", the data interpretation being carried out with the Ti-01 program but also by EDAX analysis.

The samples were examined by optical microscopy using the Olympus GX51 Inverted Optical Metallographic Microscope (Fig. 5.3) and by scanning electron microscopy (SEM) using the Inspect S apparatus, FEI equipped with the EDAX Z2e analyzer (Fig. 5.4).

Microhardness testing was performed using the Shimadzu HMV 2TE Microhardness, using a force of 980.07 mN for a duration of 10s.

5.3 Results

5.3.1 The experimental alloys obtained

Following the analysis performed with the SPECTROMAXx M device, using the Ti-01-M program, the composition values of the three alloys are those in Table V.1.

Table V.1 - Chemical composition of experimental Ti base alloys, values in weight

Chemical elements to be alloyed (wt%)	***Type of alloy***		
	Ti11Al3V2Fe	***Ti18Al7Fe5V***	***Ti6Mn2Al2VFe***
Mn	***0***	***0***	***6,59***
Fe	***2,59***	***7,81***	***1,19***
Al	***11,35***	***18,58***	***2,5***
V	***3,05***	***5,11***	***2,3***
Ti	***balance***	***balance***	***balance***

5.3.2 Microstructural analysis of the obtained alloys

After specific processing, the three alloy samples were subjected to optical and electron microscopy analysis to evaluate the structure and disposition of the constituent elements.

For the Ti11Al3V2Fe alloy, the composition of the alloying elements includes both stabilizing elements for the α phase (Al) and for the β phase. Even if the stabilizing elements of the β phase are in small quantity, below 6% by mass, the obtained alloy is bi-phasic, the α phase being predominant in the alloy structure (Fig. 5.1, Fig. 5.2).

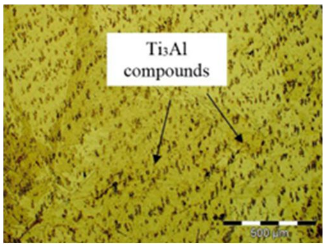

Fig. 5.1 – Optical microscopy aspect of the experimental Ti11Al3V2Fe alloy - Overview of the microstructure with the highlighting of the Ti3Al compounds.

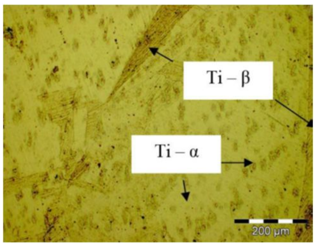

Fig. 5.2 – Details of the α and β phases in the structure of the experimental Ti11Al3V2Fe alloy, optical microscopy aspect.

The second experimental alloy has a concentration of 7.81% mass percent for Fe in the network created by Ti and Al, which leads to the transformation of the TiFe2 compounds in the alloy structure into spherical elements (Fig. 5.3). The EDAX analysis highlights the components of the structure of the experimental alloy: Ti, Al, Fe, V (Fig. 5.4).

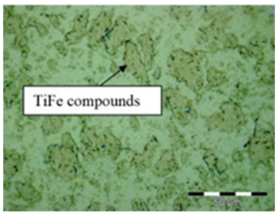

Fig. 5.3 – Optical microscopy appearance of Ti18Al7Fe5V alloy

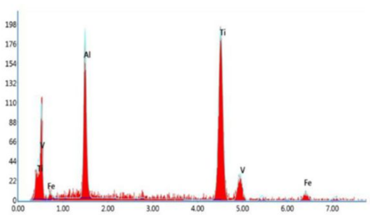

Fig. 5.4 – EDAX analysis of Ti18Al7Fe5V alloy

The addition of Mn in the composition of the experimental alloy Ti6Mn2Al2VFe (Mn 6.59% by mass), led to the separation of the round intermetallic compounds from the metal matrix represented by the α phase (Fig. 5.5). At the same time, a stabilization of the β phase is obtained, with uniformly equi-axially oriented grains and a lamellar appearance (Fig. 5.5).

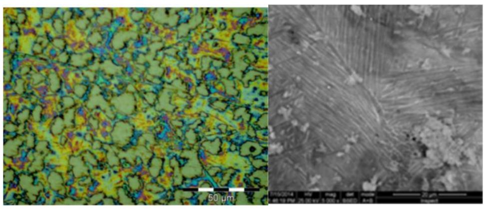

Fig. 5.5 – Optical and electron microscopy appearance of Ti6Mn2Al2VFe alloy.

5.3.3 Microhardness analysis of experimental alloys

Microhardness testing was performed using the Shimadzu HMV 2TE machine. The force applied each time was 980.07mN for 10 seconds. For each alloy under analysis, 10 measurements were made at different points of the samples, the average values being those shown in Table V.3.

Table V.3 – Microhardness measured for the three types of experimental alloys

	Ti11Al3V2Fe	***Ti18Al7Fe5V***	***Ti6Mn2Al3VFe***
Average value (HV1)	***461***	***634***	***427***

5.4 Discussions

The use of Al in the composition of Ti-based alloys has its origins in the industrial domain, and this is due to the physical properties that the alloys acquire as a result of alloying with it. Al is a stabilizing element for the α-phase of Ti alloys and among the few chemical elements that increase the temperature at which the allotropic transformation of Ti base alloys occurs [3]. Al can form a wide range of solid solutions with Ti, which vary depending on the temperature and the concentration of Al in the mass of the alloy, as can be seen from the phase diagrams for the Ti-Al complex (Fig. 5.6) [10] [11]. The solubility of Al in Ti is about 25% at the temperature of 1400°C and about 6% at room temperature [10] [11]. For these reasons, Al is considered as the main alloying element, being present in most titanium alloys (Fig. 5.6). The effect of Al in Ti base alloys is considered to be similar to that of C in Fe alloys.

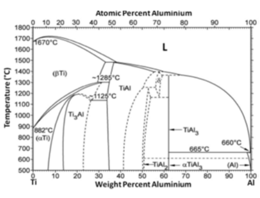

Fig. 5.6 – Diagram of the structural phases in which Ti-Al alloys can be found depending on the temperature and the mass percentage concentration of Al [10].

Exceeding the equilibrium concentration of Al (5% by mass), causes a series of secondary compounds, such as Ti3Al, to begin to separate from the solid solution. The α2 phase exerts a strong hardening effect, manifested by an increase in hardness, which reduces the plasticity of the alloy and the possibility of cold working of the alloy. Thus, it is recommended to limit the amount of Al in Ti alloys to a maximum value of 8% by mass

[12]. However, Al is an undesirable element in biocompatible alloys because Al poisonings are recognized both as occupational diseases and in patients undergoing chronic renal dialysis [13] [12] [4] [14] [11]. These aspects are also found at the level of the experimental alloys in this study.

The improvement of Ti-Al alloys can be achieved by adding V to their composition and applying thermal treatments [15], thus obtaining alloys with stabilizing elements for both the α and β phases. The mechanical properties of biphasic Ti alloys depend on the morphology of the two phases. The proportion of the lamellar structure is important because it determines increased resistance to fatigue cracking as well as good fracture toughness. During the technological processing of biphasic Ti alloys, the grain size of the lamellar phase must be maintained in the finest form.

By limiting the grain growth tendency, Fe helps to refine the grains in the microstructure of Ti alloys. Analysis of the Ti-Fe diagram shows that the following compounds can be formed:

- TiFe2 – congruent compound, with melting point at 1700 K;
- TiFe – incongruent compound, with melting point at 1650 K;
- Ti2Fe – incongruent compound, with melting point at 1358 K [16].

The most stable compound is TiFe2. The addition of stabilizing elements for the β phase in the Ti base alloys have the role of changing the proportion between the two main phases, but the α phase will still be present [17] [18] [19].

Mn was introduced in Ti base alloys, with different alloy formulas such as: Ti2Mn, Ti5Mn or Ti8Mn. The obtained alloys have good mechanical properties and an acceptable cyto-compatibility, being used as a bone substitute or alternatives to dental implants [20]. The maximum solubility of Mn in the β phase of Ti alloys is about 30% atomic percent at 1174℃, while the maximum solubility in the α phase is 0.4% atomic percent below the temperature of 882℃ [21].

In order to understand the effect on microhardness that Fe and Mn have in Ti base alloys, a comparison with other alloys in which these elements are found must be made. For comparison, alloys made experimentally in studies led by Prof. Univ. Dr. Victor Geantă and Prof. Univ. Dr. Ionelia Voiculescu (Fig. 5.7).

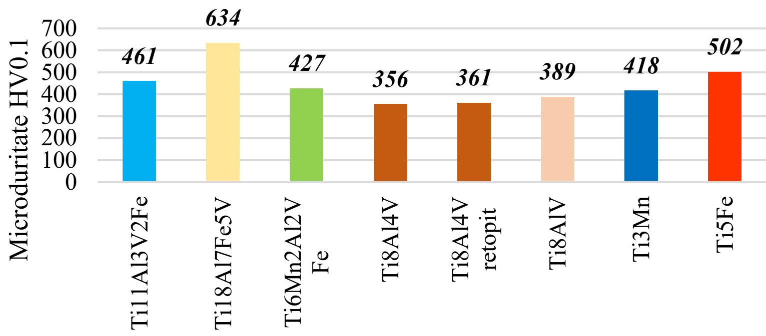

Fig. 5.7 – Comparative analysis of microhardness for different experimental Ti-base alloys

As can be seen from the graphical analysis, the introduction of Fe and Mn in the chemical composition of Ti base alloys has the effect of increasing the hardness of the alloys. This can be explained on the one hand by the hardening mechanism of solid solutions and on the other hand by the formation of hard compounds. The highest hardness value was obtained for the Ti18Al7Fe5V alloy, an alloy that has almost double the hardness compared to the Ti8Al4V alloy. This increase is determined by the addition in the composition of the alloy of an amount of Fe that exceeds the value of 5%.

And Mn has the effect of increasing the hardness of Ti base alloys in which it is included, a lesser effect than that of Fe. The values recorded for alloys containing also Mn are superior to those containing only Al (8% mass percent) and V (4% mass percent), and in addition to these, alloys with Mn are recommended for medical applications from the point of view of biocompatibility.

5.5 Conclusions

Alloying elements have different effects on the microstructure and microhardness of Ti-based alloys. For contents below 8% Al in mass percent, the alpha phases have a lamellar appearance, and above this value, the Ti3Al compounds are separated in the form of scattered islands of irregular shape.

Iron allows grain refinement and influences the spheroidization tendency of Ti-Fe compounds, contributing to matrix reinforcement.

By increasing the concentration of manganese in the Ti base alloy, the proportion of intermetallic compounds that can separate from the metal matrix, having a fine and lamellar appearance, increases. A substantial increase in microhardness in the analyzed titanium alloys promotes the use of Fe, V and Al in such alloys. These elements can act simultaneously to increase the hardness, as in the case of the Ti18Al7Fe5V alloy, having 634 HV0.1. An individual hardening effect was obtained for the Ti5Fe alloy, having a hardness of 502 HV0.1. The effect of Mn on the hardness increase was less important, producing a slight increase in microhardness from 418HV0.1 for Ti3Mn to 427HV0.1 for Ti6Mn2Al2VFe.

Chapter 6 – Biological Evaluation of MoNbTaTiZr High Entropy Alloys Used for Medical Applications

6.1 Introduction

High-entropy alloys have also begun to appear in the category of biocompatible materials, particularly based on the selection for the alloying process of elements known to be biocompatible or inert to living tissues. In this regard, there are numerous alloy formulas that have begun to be tested for use in various medical fields, such as FeMoTaTiZr, WNbMoTa, WNbMoTaV, MoNbTaTiZr [22] [23] [24] [25].

6.2 Purpose of the study

The objectives of the study are related to how bone marrow-derived mesenchymal stem cells react when placed around the obtained experimental high-entropy alloy. We wanted to evaluate the viability and proliferation of stem cells, more precisely, the assessment of the direct adhesion of the cells and their degree of survival on the surface of the experimental alloy.

6.3 Material and method

6.3.1 Obtaining the high entropy alloy

To obtain the experimental alloy, the "RAF MRF ABJ 900" Vacuum Arc Remelting Facility of the ERAMET Laboratory, Faculty of Materials Science and Engineering, Bucharest Polytechnic University, laboratory led by Prof. Univ. Dr. Eng. Victor Geantă (www.eramet.ro).

Fig. 6.1 – The base plate used to obtain the test ingots (picture from the archive of Prof. Univ. Dr. Ing. Victor Geantă).

The obtained mini-ingots were sectioned by electro-erosion and prepared for biocompatibility tests (Fig. 6.2).

Fig. 6.2 – MoNbTaTiZr ingots, high entropy alloy, obtained using the "RAF MRF ABJ 900" facility

Table VI.1 – Chemical composition of high entropy alloy, designed and obtained value

Chemical composition	Chemical element (wt%)				
	Mo	Nb	Ta	Ti	Zr
Designed	**18,86**	**18,27**	**35,56**	**9,43**	**17,88**
Obtained	**18,32**	**17,97**	**36,25**	**10,11**	**17,35**

6.3.2 Preparation of experiments to evaluate the biocompatibility and cytotoxicity of MoNbTaTiZr alloys

Isolation of bone marrow-derived mesenchymal stem cells (BDMSCs) was performed using residual bone tissue removed from the femoral head of patients with osteoarthritis, prior to insertion of a femoral prosthesis during total hip arthroplasty. The bone extraction was performed in accordance with the agreement of the Medical Ethics Committee of the Tg County Emergency Clinical Hospital. Mureș - Romania, no. 19263 / 31.07.2017, the patients involved signed the informed consent form. The method for isolation and culture of BDMSC was developed according to the method published by Gartland et al. 2012 [26].

6.4 Results

6.4.1 Cell viability and proliferation on alloy samples

Bone fragments cultured as E1 explants yielded BDMSCs, which after 4 weeks reached 90–100% confluence, as seen in Fig. 6.4 and Fig. 6.5. The explant culture technique yielded a sufficient number of cells by splitting cells at a ratio of 1:2 during subculture from passage P0 to passage P1 and after thawing cryopreserved cells at -140°C from passage P1 at passage P2. BDMSCs from P2 and NHFs from P28 were used to test the biocompatibility of MoNbTaTiZr alloys. Biocompatibility testing was performed with BDMSCs from a single 48-year-old male patient, as bone fragments taken from this patient provided approximately $6x10^6$ mesenchymal stem cells in P2.

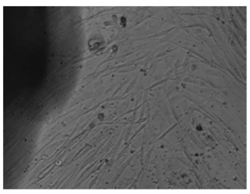

Fig. 6.3 – Bone fragments cultured as explant, BDMSC from E1 explant, achieving 90–100% confluence. (Leica DMi8 microscope, 100x magnification)

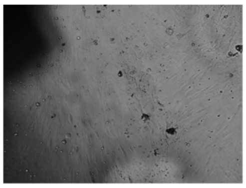

Fig. 6.4 – Bone fragments cultured as explant, BDMSC from E2 explant at confluence. (Leica DMi8 microscope, 100x magnification)

Images illustrating the progression of BDMSCs and NHFs after 5 days in culture on the surfaces of the MoNbTaTiZr alloy and on the bottom of the wells are shown in Fig. 6.3 – 6.4. Analysis of cell viability and proliferation was performed with a Leica DMi8 microscope, in wide-field fluorescence, following cell staining with propidium iodide and Calcein AM. After microscopic analysis and image acquisition, adequate viability can be observed by the exclusive presence of green fluorescence emission of Calcein after hydrolysis of the acetoxymethyl ester by active intracellular esterases, which occurs only in living cells. In the images that were acquired, viable BDMSCs with normal morphology, namely fibroblast-like cells or spindle-shaped cells and large, flattened cells, can be seen. Neither abnormal, round cell morphologies nor red emission due to intracellular penetration of propidium iodide into damaged cells were detected. After 5 days of culture, proliferating cell groups can be observed on the surface and near all alloy samples seeded with BDMSC, with a confluence that can be estimated at 50-60%. BDMSC behaved similarly in control wells, where cells were grown under the same culture conditions but in the absence of alloy samples.

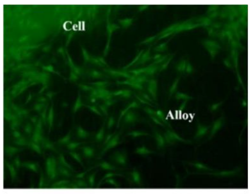

Fig. 6.5 – Viability, adhesion and proliferation of BDMSCs on the surface and near MoNbTaTiZr alloy samples after 5 days in culture, fluorescence acquisition, 100x magnification;

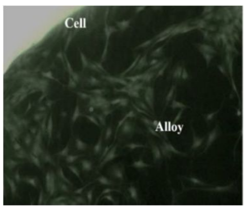

Fig. 6.6 – Viability, adhesion and proliferation of BDMSCs on the surface and near MoNbTaTiZr alloy samples after 5 days in culture, phase contrast acquisition, 100x magnification – same field of view as Fig. 6.5.

6.4.2 Cytotoxicity evaluation in the presence of MoNbTaTiZr alloy by determining Lactate Dehydrogenase activity in culture media

In the presence of cytotoxic compounds, living cells can stop growing and dividing. Moreover, cells can die by necrosis or apoptosis. Cells undergoing necrosis lose their membrane integrity and release intracellular molecules into the environment. Necrosis is usually triggered by external factors such as toxic chemicals. Cells undergoing apoptosis are characterized by cytoplasmic shrinkage and DNA cleavage into apoptotic bodies. In such cases, when cell membranes are damaged in any way, lactate dehydrogenase (LDH), a soluble enzyme found in living cells, is released into the extracellular environment, so the presence of this enzyme in the culture medium can be used as a marker of cell death in a mixed population of healthy and damaged cells. The increase in LDH activity in cell culture media is proportional to the number of damaged cells.

Cytotoxicity assays assessing LDH activity in culture media from NHF showed increased LDH activity between culture media from day 5 to day 10 in control wells (p=0.0079), where cells were cultured without alloy, as well as in the wells where the cells were cultured in the presence of the alloy (p=0.0079), with the same statistical significance (Fig. 6.7). Thus, this increase in LDH activity in both control and alloy wells is not due to cytotoxicity of the alloys. The presence of the alloy sample did not lead to certain cytolysis phenomena. The same observations can be made for the increase in LDH activity from day 5 to day 10 in the medium of control wells (significant increase with p=0.0065) and wells where BDMSCs were grown on alloy surfaces (same significant increase with p= 0.0065) (Fig. 6.8).

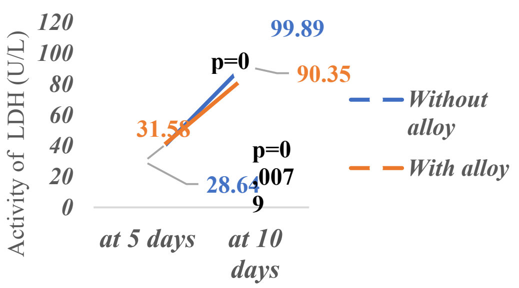

Fig. 6.7 – Evolution of median values of LDH activity in NHF cultures from day 5 to day 10 in the presence and absence of alloy samples.

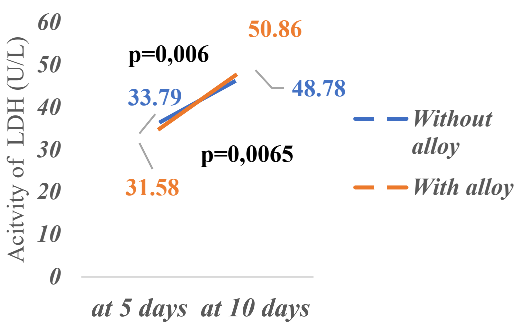

Fig. 6.8 – Evolution of median values of LDH activity in BDMSC cultures from day 5 to day 10 in the presence and absence of alloy samples.

Higher values of LDH activity are found in the wells where NHF was cultured, probably due to the more intense proliferation of this cell line and the appearance of the growth inhibition phenomenon after high-speed proliferation. One of the causes of apoptosis and necrosis in cell cultures is the excessive growth of cells on a given culture surface. Passage of cell cultures should be performed in the log phase of cell growth (the period of exponential increase in cell number), not allowing a confluence exceeding 80-90%. In wells that were seeded with NHF, cells reached 100% confluence, with areas of overlap (overcrowding) of cells in the well at 10 days, even in contact with the alloy samples. Given this intense proliferation of NHF, the perspective would be to grow NHF at a lower density of 10-20 $x10^4$ per well.

6.5 Conclusions

High entropy alloys of the MoNbTaTiZr system, due to their mechanical and biomedical properties, have some use in the medical field, both for medical instrumentation and surgical applications.

The technological process of obtaining samples from the MoNbTaTiZr system in the VAR vacuum arc melting facility attests to the optimal option for obtaining them. Material losses are minimal, and EDS analyzes attest to a good agreement between the designed and obtained compositions.

Microscopic investigation of cell behavior after culturing mesenchymal stem cells and normal human fibroblasts in direct contact with MoNbTaTiZr alloy samples demonstrates the biocompatibility of this type of alloy. Both types of cell lines showed initial adhesion followed by active spreading in contact with the alloy samples. Mesenchymal stem cells had good viability and proliferated to 80-90% confluence within 10 days of assay, both in control wells - in the absence of alloy and in wells with alloy samples. Similarly, proliferation to 100% confluence was observed for the human fibroblast line both in the vicinity of the alloy and on its surface with good maintenance of viability.

Cytotoxicity assays by determining LDH activity in culture media showed a similar increase in LDH activity in both control wells where cells were cultured without alloy and wells where cells were cultured in the presence of alloy. The presence of alloy samples did not lead to a certain addition of cytolysis phenomena (cytotoxicity).

Chapter 7 – Conclusions and personal contributions

Following this study, the following conclusions can be drawn:

- Experimental alloys based on Ti can be obtained in laboratory conditions by alloying with Nb, Zr and Ta;

- Due to the method of obtaining the composition is not homogeneous, there are areas of concentration of alloying elements;

- Changing the composition (adding or not Ta) as well as changing the proportions in which the allied elements are found lead to changing the values of microhardness and shear strength;

- Reducing the amount of Zr leads to a decrease in microhardness and shear resistance, the alloy thus obtained being more malleable (compared to Ti1 and Ti3) when forces are applied.

- Alloys with similar or even better hardness than those currently used in dental practice can be obtained.

- The alloys obtained (Ti1 and Ti3) meet the minimum criteria for the shear strength of the metal-ceramic bond even if a standard program not adapted to their chemical composition was used.

- Iron allows grain refinement and influences the spheroidization tendency of Ti-Fe compounds, contributing to matrix reinforcement.

- By increasing the concentration of manganese in the Ti base alloy, the proportion of intermetallic compounds that can separate from the metal matrix, having a fine and lamellar appearance, increases.

- A substantial increase in microhardness in the analyzed titanium alloys promotes the use of Fe, V and Al in such alloys. These elements can act simultaneously to increase the hardness, as in the case of the Ti18Al7Fe5V alloy, having 634 HV0.1. An individual hardening effect was obtained for the Ti5Fe alloy, having a hardness of 502 HV0.1.

- The effect of Mn on hardness increase was less important, producing a slight increase in microhardness from 418HV0.1 for Ti3Mn to 427HV0.1 for Ti6Mn2Al2VFe.

- The high entropy alloy based on the elements Mo, Nb, Ta, Ti, Zr provides good cell adhesion and promotes active cell spreading with a confluence between 80% and 100% after 10 days of analysis.

- The presence of the alloy does not increase the rate of cytolysis phenomena (It does not increase cytotoxicity).

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List of scientific papers published in the framework of doctoral research

1. **S Tudoran**, I Voiculescu, V Geantă, P Vizureanu, I Mârza Roşca, I Pătraşcu, B M Gălbinaşu and R Ciocoiu, ”Effects of the chemical composition on the microstructural characteristics of Ti-Nb-Ta-Zr alloys”, IOP Conference Series: Materials Science and Engineering, Volume 572, International Conference on Innovative Research - ICIR EUROINVENT 2019 16–17 May 2019, Iasi, Romania, https://**DOI**/10.1088/1757-899X/572/1/012022

The article is written starting from the data presented in Study 1 of the Doctoral Thesis (Chapter 4 – pages 45-49).

2. **Tudoran S**, Ciocan LT, Spinu T, Galbinasu BM, Patrascu I, Vasilescu VG. Adhesion evaluation of dental ceramics sintered on novel titanium alloys. *Ro J Stomatol*. 2023, Vol. 69, Issue 4, pag.198-207. *https://doi.org/10.37897/RJS.2023.4.2*

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3. VICTOR GEANTA, IONELIA VOICULESCU, **STEFAN TUDORAN**, ”Effects of Fe and Mn on Microstructure and Microhardness of Titanium Alloys”, Revista de Chimie, 2020, Vol. 71, Issue 4. Pag 87-94, *https://doi.org/10.37358/RC.20.4.8046*

The article is written starting from the data presented in Study 2 of the Doctoral Thesis (Chapter 5 – pages 68-84).