NEW TITANIUM β-ALLOYS FOR DENTAL IMPLANTOLOGY AND THEIR LABORATORY-BASED ASSAYS OF BIOCOMPATIBILITY

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ABSTRACT

In this study, we focused on biological aspects of the applicability of modern titanium alloys in dental medicine. Overall, 34 new titanium β-alloys with different amounts of niobium (Nb), molybdenum (Mo), tantalum (Ta), vanadium (Va), and iron (Fe) were tested. Their suitability for possible use in dental implants was evaluated with special regard to potential toxicity. To evaluate biocompatibility of β-alloys, we applied numerous biological tests focused on cell growth, cell adherence, cell dilatation, evaluation of toxicity, tests of chromosomal aberrance, and other parameters of biocompatibility. The results revealed that, due to elution of particles of intermetallic TiFe phase and consequent release of Fe ions, TiAlFe alloys were not suitable for dental implants. TiAlNb and TiTa alloys exhibited the required biocompatibility parameters. The TiAlV alloys were ranked as biotolerant. It might be concluded that TiMo alloys were acceptable for the production of dental implants but less suitable than TiAlNb and TiTa alloys.

INTRODUCTION

There is a relatively large time gap between the invention and the development of a new dental material and its application in clinical practice. Within this time, numerous material-related and laboratory-based tests, as well as preclinical and clinical trials, are done. The length of the period, however, might be shortened if effective multidisciplinary research is applied. Such research requires close collaboration among specialists from the fields of material engineering, dental implantation surgery, and stomatology. In our study aimed at the period of such tests in newly-developed alloys, we created a network of professional institutions comprising a stomatological clinic, a stomatological research centre, and several commercial companies in the field of biotests,

implantation surgery, and orthopaedics. In our study, we focused on the newly-developed titanium β-alloys, Ti38Nb and Ti35Nb6Ta, as well as on those with niobium (Nb), molybdenum (Mo), tantalum (Ta), vanadium (Va), and iron (Fe) tested for future use in dental implants. Due to the low value of Young modulus and relatively easy forming, even cold processing, the β-alloys have good characteristics for prospective application in dental implantology. Therefore, future dental implants would be prepared using routine foundry technologies. Undoubtedly, many new applications of β-alloys will appear in future dentistry [1]. It is, therefore, necessary to define requirements for mechanical characteristics in such materials and implants. First of all, mechanical and physical properties of the new cast material must be determined and compared to those of formed materials. Then, their possible application must reflect their performance in dental implants exposed to different load in a jaw. Especially the fatigue load might be a critical issue in some applications of dental implants. In this respect, it is highly desirable to compare the fatigue load in formed and cast alloys. Specifically for β-alloys, it is necessary to keep their properties constant, in spite of the fact that they may vary according to their complex microstructure, number and amount of alloy ingredients. The alloys may be deformed mainly by sliding or twinning and/or combination of the two. Additionally, the alloys may have unsuitable martensic structure. The fatigue properties of the alloys, especially the probability of microcrack initiation and their further development, depend strongly on alloy microstructure, composition, and temperature of processing. They may affect substantially the final properties of the alloy and the dental implant.

In our study, we focused on biological aspects of the applicability of modern titanium alloys in human medicine, dental implantology in particular. Within the last decades, numerous titanium alloys have been developed. These alloys possess suitable mechanical properties and a higher biocompatibility than traditional titanium-based materials [2]. In the Czech Republic, the majority of titanium dental alloys are of a high degree of purity abbreviated as Grade 2. For such purity, the maximum Fe content allowed is 0.25 % of weight. It is 0.20 %, and 0.08 % for carbon. The rigidness of such grade is within the range of 390–540 MPa. Formed materials obviously show rigidness at the upper limit. New materials could therefore show higher rigidness. They could also fit the high requirements for optimum biomechanical and biological interactions. The new materials should minimise fatigue and corrosion [3], which are the main reasons for implant rupture. Local load caused by chewing forces and the activity of oral cavity liquids may induce damage. It might be demonstrated as fretting damage, corrosion, and surface tension of implants. Generally, low pH and the resulting electrochemical changes in the oral cavity [4] may cause damage to dental implants, too. Normal values (pH about 7.0) may be decreased mainly in the close neighbourhood of haematomas. Interstitial liquids, especially chlorides, are the most aggressive agents to implants. They may induce pitting, corrosion along margins, crack corrosion, etc. Therefore, studies of the response of dental implant materials to a variety of mechanical, biochemical, and biological factors are highly desirable.

In our study, we focused on the behaviour of titanium alloys in biological environment and evaluation of corrosion and the toxic effect of eluted metal ions. Another aim was to analyse the likely reasons for biological rejection of titanium alloys. The aim of our study was to perform a series of tests of biocompatibility of β-alloys with special regard to those of Ti38Nb and Ti35Nb6Ta grade. A further aim was to point out several alloys with the best properties and thus most suitable for clinical dental applications. Our working hypothesis was that a change in the relative proportion of Ni, Mo, Ta, Va, and Fe in dental implants of β-alloys may cause substantial differences in their properties and suitability for application in human medicine. For this purpose, we selected several biological tests that are used in the evaluation of biocompatibility of materials. The biological tests were focused on the following fields: cell suspension growth in an elution from tested alloys, adherence, dilatation (spreading), and toleration tests. Last but not least, a test of atypical mitosis and a test of chromosomal aberrance were applied.

MATERIALS AND METHODS

Laboratory-based preparation of titanium alloys Titanium alloys were prepared in an electric arc furnace Leybold-Heraeus L2004 supplemented with a pump A2DS150 and an exhauster Roots RP1800. The alloys were smelted in water-cooled crystallisers in vacuum (10^{-2} to10^{-3} mbar) or under low pressure atmosphere of an inert gas (He or Ar, 300–400 mbar). The smelting was performed either by a melting electrode in vacuum or a non-melting tungsten electrode under He/Ar atmosphere. The power source of the whole system was capable of a maximum current of 2 500 A. For the typical melting process, 1200 A was typically used.

Several titanium alloys with different contents of admixtures were prepared. The relative shares of the admixtures were analysed by a certified laboratory (Chemopetrol, Litvínov, CZ) using standard chemical analyses for trace element determination. Table 1 summarises the alloys prepared and tested in our study.

Sample preparation for biological testing

From the laboratory-prepared alloys, small pieces were cut and subjected to a metallographic analysis and rigidness measurements. The samples were analysed in the initial

Table 1

Titanium alloys and their chemical composition. For trace elements, only the alloyed ones are listed. Other elements important for the alloy properties are listed as well. Key to abbreviations: Abbr. – abbreviation, Al – aluminium, V – vanadium, Ta – tantalum, Nb – niobium, Fe – iron, Mo – molybdenum, Zr – zirconium, O – oxygen, N – nitrogen

Alloy	Abbr.	Concentration [% weight]									
		Al	V	Ta	Nb	Fe	Mo	Zr	O	N	
Ti6Al2V	**V1**	6.56	1.75	-	-	-	-	-	0.119	0.028	
Ti6Al4V	**V2**	6.70	3.71	-	-	-	-	-	0.145	0.022	
Ti3Al2,5V	**V5**	3.26	2.21	-	-	-	-	-	0.091	0.009	
Ti6Al6V	**V3**	6.59	5.63	-	-	-	-	-	0.140	0.037	
Ti6Al8V	**V4**	6.85	7.85	-	-	-	-	-	0.133	0.036	
Ti6Al2Nb	**N1**	6.11	-	-	2.42	-	-	-	0.141	0.041	
Ti6Al4Nb	**N2**	5.91	-	-	4.84	-	-	-	0.095	0.012	
Ti6Al6Nb	**N3**	5.85	-	-	7.16	-	-	-	0.145	0.031	
Ti6Al7Nb	**N4**	5.93	-	-	8.36	-	-	-	0.149	0.033	
Ti6Al1Nb	**N5**	5.90	-	-	10.45	-	-	-	0.083	0.014	
Ti5Al1Fe	**F1**	5.15	-	-	-	1.10	-	-	0.150	0.037	
Ti5Al2,5Fe	**F2**	5.30	-	-	-	2.69	-	-	0.121	0.031	
Ti5Al4Fe	**F3**	5.38	-	-	-	4.22	-	-	0.131	0.036	
Ti5Al6Fe	**F4**	5.11	-	-	-	7.38	-	-	0.149	0.032	
Ti5Al8Fe	**F5**	5.00	-	-	-	10.18	-	-	0.137	0.030	
Ti5Ta	**M1**	-	-	5.39	-	-	-	-	0.178	0.043	
Ti10Ta	**M2**	-	-	9.83	-	-	-	-	0.185	0.035	
Ti15Ta	**M3**	-	-	14.80	-	-	-	-	0.192	0.031	
Ti20Ta	**M4**	-	-	19.70	-	-	-	-	0.154	0.024	
Ti25Ta	**M5**	-	-	24.39	-	-	-	-	0.198	0.020	
Ti15TaZr	**MZ3**	-	-	14.70	-	-	-	5.61	0.076	0.0058	
Ti30Ta	**M6**			28.95					0.089	0.0075	
Ti35Ta	**M7**			33.81					0.120	0.023	
Ti30Nb	**N6**				31.81				0.071	0.015	
Ti5Mo	**P1**						5.16		0.170	0.055	
Ti10Mo	**P2**						9.96		0.167	0.039	
Ti15Mo	**P3**						15.12		0.183	0.044	
Ti20Mo	**P4**						19.78		0.185	0.055	
Ti25Mo	**P5**						24.90		0.155	0.038	
Ti30Mo	**P6**						29.41		0.148	0.032	
Ti35Mo	**P7**						34.49		0.164	0.034	
Ti15Mo5Zr	**PZ3**						14.79	5.24	0.150	0.032	
Ti25Mo5Zr	**PZ2**						24.48	5.26	0.0077	0.0060	
Ti15Nb8Zr	**ZN1**				15.51			8.64	0.113	0.0098	

state after melting and at different states after annealing at 650 °C (15 min, air, abbreviated as Z in the following text), 850 °C (10 min, furnace, abbreviated as R), and 950 °C (10 min, furnace, abbreviated as P), respectively. For each sample and annealing type, microstructure of the alloy was evaluated. Microstructure was evaluated on a Lucia (NIKON) analysis system. The rigidness of the alloys was measured with a Vickers HV10 rigidometer. For selected samples, chemical analyses of phases were done using the microanalyser of a JOEL scanning electron microscope. For biological tests, cylinders of Ø 8x3 mm in diameter were prepared from the alloys.

Biological assays

To assess the interactions between the alloys and the biological material, several biological tests were applied. First, we used a test of cell growth in the elution of tested alloys for the evaluation of potential toxicity. Heteroploid cell lines were exposed to 4 treatments (concentrations), and positive and negative controls, respectively. The number of cells was counted in 1 ml of the elution every 24 h for 5 d. In the test of cell adherence, a cell suspension of the same lines was spread over the tested alloys and exposed for 24 h. Then, the cells were fixed in glutaraldehyde and prepared for analysis by a critical-point drying. The morphology of the adhered cells was evaluated

using a SEM (Vega Tescan microscope). The values obtained were compared to a standard surface (cover glass) and the relative number of adhered cells (an alloy/glass standard) was calculated. A dynamic dilatation (spreading) test of the cells was applied to evaluate the number of dilated cells adhered to a substrate with a membrane spread over the substrate after 6 h of exposition to the elution [5]. The other test was the tolerance test of a cell monolayer. A piece of the tested alloy was put into a cell culture monolayer in a cultivation flask. After 48 h exposition, the size of the death cell area was evaluated. A similar test was the test of cell tolerance in suspension to the tested material. The material of the tested alloy was put into a cultivation flask and then a cell suspension was added. The size of the area on the surface of the tested material on which adherence was inhibited was evaluated.

Induction of atypical mitosis was tested in a cell suspension inoculated to a cover glass and exposed for 72 h. Similarly to the cell growth test, 4 different concentrations of elution, and positive and negative controls were used. After the exposition, the number of metaphases, the number of cells possessing more than one nucleus, the number of multipolar divisions, and the mitotic index were evaluated. If there was more than 10 % of mitoses, the tested material was considered mutagenic. A clastogenic test, i.e. a test of induction of structural chromosomal aberrance, was applied to human leucocytes. After 72 h exposition, structural and numeric chromosome aberrances were counted and compared to positive and negative controls, respectively. To stop metaphase, colcemide was added to the cell monolayer 3 hours before harvesting. For details of the method see e.g. [6]. The last test that we applied was the test of chromosomal aberrance in human peripheral lymphocytes. In the test, fully heparinised blood is cultivated in the presence of PHA. Metaphased chromosomes were prepared. After 72 h exposition, chromosomal breaks were evaluated in 200 mitoses observed. In this case (e.g. the test on human leukocytes) more than 5 % of aberrant mitoses was assumed as a mutagenic effect.

RESULTS AND DISCUSSION

The screening test and two basic tests of cytocompatibility of selected pure metals showed that only four of the metals tested exhibited high biocompatibility (Table 2). Apart from Ti, also Ta, Nb, and Zr were highly biocompatible and thus potentially prospective for biocompatible alloys. Ti and Zr have similar properties. They are, therefore, substitutable. Ni and Ta also have similar properties. They can, therefore, be used in alloys with Zr and Ti, in which they enlarge the β phase. If their proportion was more than 30 %, then the resulting β alloy hardened by globular particles of the α phase. TiNb, TiTa alloys or their ternary combination are suitable for cold welding. This represents a great technological advantage in comparison to the traditionally used Ti6Al4V alloys. Moreover, TiNb and TiTa alloys do not form intermetallic phases, the properties of which differ from pure metals. On the other hand, there are some disadvantages. Tantalum has a high melting temperature, which is complicating for metallurgical processing. Therefore, alloys with Ni are preferred. In alloys with Nb, a proper share of Nb and processing is required to reach the β phase. When low Nb concentrations are used, then an undesirable martensite structure is reached (see Figure 1 left), characteristic of numerous intermartensite

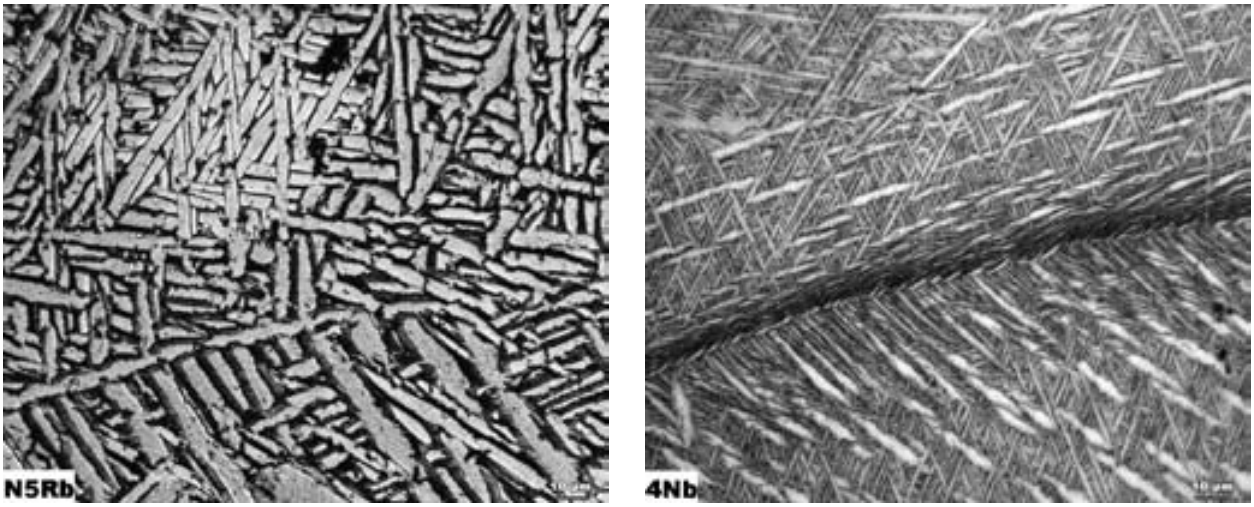

Figure 1
Microstructure of Ti6Al7Nb alloy after casting (left) and Ti6Al10Nb cooled 10 min at 850 °C/10 (right).

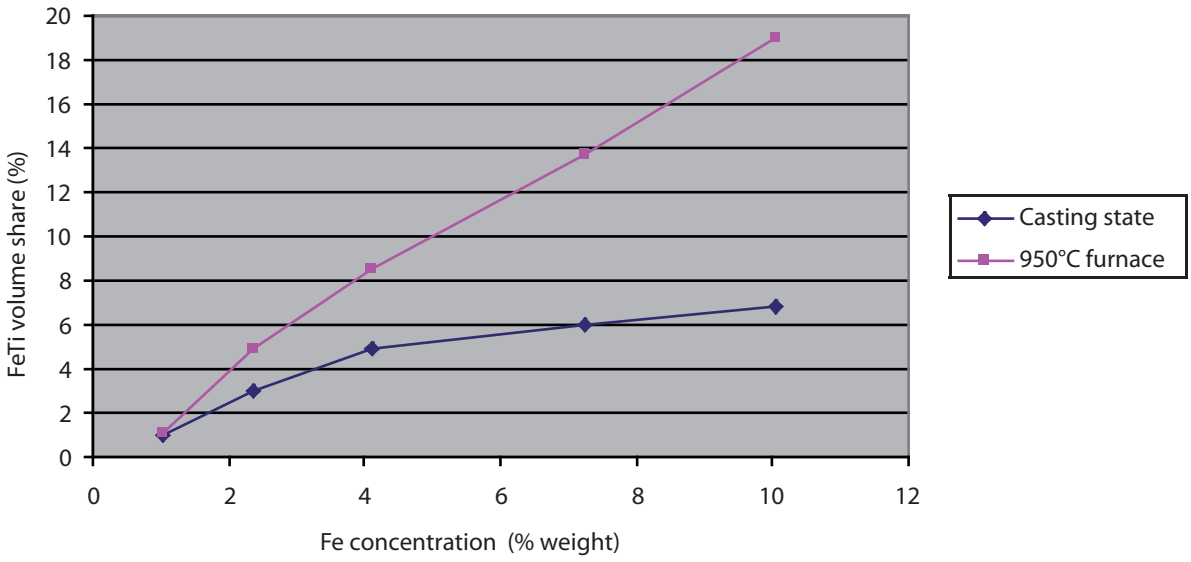

Figure 2
Relation of the volume share of intermetallic TiFe phase to Fe concentration and heat processing

spaces untransformed into the β phase. After slow cooling of the alloy, a typical combined α+β structure is reached (Figure 1 right). To achieve a proper β-phase alloy, higher Nb concentrations must be used (Ti35Nb).

A test of cell growth in the elution showed that the kinetics were of the same shape (data not shown) in TiAlFe, TiAlNb, and TiTa alloys. Similar growth curves were also found in an earlier study focused on titanium implants covered with a surface Cr-Co alloy [7]. For TiAlFe alloys, the number of cells found for particular lengths of exposition was the same, slightly lower if compared to a positive etalon. From this point of view, Fe alloys do not exhibit any toxic effect. A dilatation test showed a reduced number of dilated cells. The reduction was more pronounced in slowly cooled alloys. Moreover, a test of tolerance showed that around TiAlFe alloys a zone of toxic effect was formed. These results showed that TiAlFe alloys were not suitable for dental implants. The reason is the likely elution of particles of the intermetallic TiFe phase and release of Fe ions from the phase (see Figure 2).

Cell adherence to TiAlFe alloys was higher when the alloys were annealed at 950 °C and cooled in a furnace. In comparison to other metals in the alloy, TiAlFe exhibited lower values of cell adherence. A high proportion of the intermetallic phase reduced the number of atypical mitoses. We can, therefore, summarise that Fe caused negative effects in cells. A carcinogenic effect cannot be therefore excluded. TiAlFe alloys have poor biocompatibility and thus cannot be recommended for dental implants.

The results obtained in biological tests with TiAlNb alloys showed that they were very suitable for dental implant production. They exhibited the required biocompatibility parameters, even if the proportion of Nb was 35 %. It means that the binary system TiNb allows to prepare biocompatible β-alloys that would not be shaped under cool conditions. Generally, TiAlNb alloys might be recommended for the production of dental implants.

A biological test of TiAlV alloys resulted in slightly negative parameters. The TiAlV alloys are, however, still ranked as biotolerant. Moreover, some anomaly was observed, such as creation of fibrous cell structures and abnormalities of cell membranes. These abnormalities will be further analysed in detail in a separate paper. It can be stated that V-alloyed materials are acceptable and have recently been frequently used for dental implant production. They are, however, less suitable. There is the probable effect of V toxicity, in spite of the fact that V is well fixed in compact solution in TiAlV alloys.

TiTa alloys showed either very good biocompatibility or at least tolerance. The compatibility was high even when the Ta proportion was above 25 %. The results of all the tests indicated that Ta-alloyed materials were very suitable for dental implant production. It means that the binary system TiTa allows to prepare biocompatible β-alloys that would not be

Table 2
Summary of biocompatibility of metals presented in the alloys tested

Element	Dilatation test	Adherence test	Screening	Overall evaluation
Al	tolerant	tolerant	toxic	tolerant
Cu	toxic	toxic	toxic	toxic
In	toxic	toxic	tolerant	toxic
Mo	tolerant	tolerant	tolerant	tolerant
Nb	compatible	compatible	compatible	compatible
Pb	toxic	toxic	toxic	toxic
Ta	compatible	compatible	compatible	compatible
Ti	compatible	compatible	compatible	compatible
V	tolerant	toxic	tolerant	tolerant
Zn	toxic	toxic	toxic	toxic
Zr	compatible	compatible	compatible	compatible

shaped under cool conditions. TiTa alloys may therefore be recommended for the production of dental implants. Moreover, recent *in vitro* studies focused on the cytotoxicity of TiTa alloys [8, 9]support the idea of application of such materials in dental implantology.

A biological test of TiMo alloys showed somewhat lower biocompatibility than TiAlNb and TiTa alloys. However, TiMo alloys were evaluated as tolerant. It might be stated that TiMo alloys are acceptable for the production of dental implants but less suitable than TiAlNb and TiTa alloys.

Concluding remarks

Biological tests and controlled experiments performed *in vitro* undoubtedly provide valuable information on the biocompatibility of β titanium alloys. Such testing may point out important factors directly and indirectly involved in the process of dental implant functioning in patients. Recently, numerous pure titanium and α and β titanium alloys have been tested for the use in dental implants [10]. However, the rigidity of α and β type titanium alloys is still considerably greater than that of the cortical bone [11], although the rigidity of titanium alloys is less than that of Co-Cr type alloys and stainless steels used for biomedical applications. Therefore, the latest trend is to develop low-rigidity β-type titanium alloys. In such a material, a strongly limited or zero presence of toxic and allergic elements is a necessity. Such titanium alloys should have, apart from excellent mechanical properties and workability [12], also a high biocompatibility.

From the experimental results it can be seen that among the alloys tested, TiAlNb and TiTa alloys are potentially highly desirable for clinical use since they exhibit the best parameters in all the biologically-related features tested. Titanium alloys used in this study showed high cell adherence, similar to the results of the recent adsorption study [13, 14]. The biocompatibility of the beta titanium alloy might be excellent similarly as found in other β-alloys with Ni, Ta, and Zr [15]. In spite of the fact that there is only limited knowledge on long-term studies of titanium alloy effects in the oral cavity and thus on the incidence of sensitivity to titanium in the general population, we may recommend TiAlNb and TiTa alloys for clinical use. Their susceptibility to fretting corrosion and potential negative effects, such as small-scale debris production resulting in cellular reaction and osteolysis [16, 17, 18], will be the subject of our further study. It has been demonstrated in our earlier studies [19] that pure titanium dental implants did not increase the number of chromosomal aberrance. The results of our present study with β titanium alloys support this information. It may be concluded that β titanium alloys undoubtedly will enlarge the set of materials used at the Department of Stomatology, Masaryk University, Brno (for overview see e.g. [20]). However, careful preclinical testing will have to precede clinical application of dental implants made of β-alloys.

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