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Research article
Научная статья

Effect of alloying with a second components on the biocompatibility and mechanical properties of Ti–Mo alloys

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Abstract: This paper presents the results of a study on two titanium-based alloys — Ti–10wt.%Mo and Ti–15wt.%Mo — aimed at assessing their potential for use as base materials in implantable medical devices for osteosynthesis. The alloy samples were examined in three conditions: as-fabricated, after annealing at 1000 °C, and after high-pressure torsion. The microstructure of the alloys was analyzed using scanning electron microscopy and *X*-ray diffraction. The Young's modulus, microhardness, and nanohardness values were measured, and the effect of the alloys on the viability and surface adhesion of human multipotent mesenchymal stromal cells during *in vitro* incubation was investigated. Comparative analysis of the obtained results revealed that the annealed Ti–15wt.%Mo alloy sample is the most promising candidate for orthopedic applications, as it exhibits an optimal combination of good biocompatibility, enhanced stimulation of cell adhesion, and relatively low microhardness (283 HV) and Young's modulus (106 GPa).

Keywords: titanium–molybdenum alloys, biocompatibility, cell adhesion, phase transformations, high-pressure torsion, nanoindentation, heat treatment.

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Влияние легирования второй компонентой на биосовместимость и механические свойства сплавов Ti–Mo

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Аннотация: Представлены результаты исследования двух сплавов на основе титана: Ti–10мас.%Mo и Ti–15мас.%Mo – для оценки перспектив их использования в качестве основы имплантируемых медицинских изделий для остеосинтеза. Образцы сплавов были изучены в трех состояниях: исходном (после изготовления), после отжига при температуре 1000 °C и после кручения под высоким давлением. Была исследована микроструктура сплавов с помощью сканирующий электронной микроскопии и рентгеноструктурного анализа. Были измерены значения модуля Юнга и микро- и нанотвердости сплавов, а также изучено влияние сплавов при инкубации *in vitro* на жизнеспособность и поверхностную адгезию мультипотентных мезенхимальных стромальных клеток человека. Сравнительный анализ характеристик исследованных образцов показал, что наиболее перспективным для использования в качестве основы ортопедических изделий является отожженный образец сплава Ti–15мас.%Mo, который оптимально сочетает хорошую биосовместимость, активную стимуляцию клеточной адгезии и низкие значения микротвердости (283 HV) и модуля Юнга (106 ГПа).

Ключевые слова: сплавы титан–молибден, биосовместимость, клеточная адгезия, фазовые превращения, кручение под высоким давлением, наноиндентирование, термообработка.

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Introduction

Metallic implants play a dominant role as structural biomaterials in reconstructive surgery, particularly in orthopedics, and in recent years have also been applied to non-osseous tissues such as blood vessels. These implants are continuously being modernized and improved, and numerous review papers have been published on the materials currently available [1—4]. Among these, β-titanium alloys occupy a special position as promising biomaterials intended to replace first-generation α-titanium alloys, commercially pure titanium, and the VT6 alloy.

Although titanium alloys have stood the test of time and are now widely used for various orthopedic and dental purposes [1], ongoing research seeks to modify their composition and surface characteristics to develop materials with the most favorable combination of mechanical and chemical properties. Owing to the unique properties of titanium, the elastic modulus of titanium alloys can be tailored to approach that of bone, primarily through alloying with elements that alter the volume fractions of structural phases in the material [2].

The interest in Ti—Mo alloys [5—9] as promising materials for biomedical applications has persisted for more than three decades. Alloying titanium with molybdenum imparts high strength and a low elastic modulus to the material.

The choice of Ti—10wt.%Mo and Ti—15wt.%Mo alloys for this study was guided by the following considerations. First, titanium is characterized by extremely low toxicity to the human body, high corrosion resistance, and resistance to biodegradation, as confirmed by the long-standing clinical use of titanium and its alloys in medicine. Second, molybdenum is a β-phase stabilizing element with low toxicity; its content in the range of 15—20 wt. % can reduce the elastic modulus and align the mechanical properties of the alloy with those of human bone tissue. An important goal of implant—tissue interaction is to prevent corrosion or degradation, bone destruction, physiological changes, or implant instability. Titanium alloys are biologically inert; however, modification of surface morphology (roughness), wettability, and other surface parameters can enhance the adhesion of connective tissue cells, particularly osteogenic cells. Titanium and its alloys spontaneously form a very stable oxide layer that separates the alloy from adjacent tissues and provides excellent corrosion resistance [10].

This study addresses not only the improvement of the mechanical properties of the investigated alloys through various processing methods but also the evaluation of how these treatments affect the biocompatibility of Ti—Mo alloys.

One of the material processing techniques capable of significantly altering both the mechanical properties [11—13] and the microstructure and phase composition [13; 14], of the alloy is severe plastic deformation [15—21]. In this work, one such technique — high-pressure torsion (HPT) — was applied to evaluate the influence of this processing method on the biological activity of the alloys.

The aim of this study is to investigate the effect of alloying element content and processing method (heat treatment and high-pressure torsion) on the mechanical properties and biocompatibility of Ti—10 wt. % Mo and Ti—15 wt. % Mo alloys.

Materials and methods

Ingots of Ti—10wt.%Mo and Ti—15wt.%Mo alloys were produced by vacuum arc melting of the components in a high-purity argon atmosphere using a water-cooled copper mold. The purity of alloying elements was at least 99.9 wt. %. To ensure chemical homogeneity, the ingots were inverted and remelted at least ten times. The melting process was carried out using an Arc-Melting AM200 unit (Bodelshausen, Germany). The resulting ingots, 10 mm in diameter, were cut into discs 0.7 mm thick. The first series of samples was studied in the as-fabricated condition, the second after heat treatment (HT) at 1000 °C for 24 h, and the third after HT followed by high-pressure torsion (HPT). HPT processing was performed at room temperature: five revolutions under a pressure of 7 GPa at a rotation rate of 1 rpm using a computer-controlled Bridgman-type anvil apparatus (W. Klement GmbH, Lang, Austria). After HPT, the sample thickness was 0.35 mm. The microstructure and phase composition of all samples were analyzed. The phase composition, phase fractions, and lattice parameters were determined using *X*-ray diffraction (XRD). Diffraction patterns were recorded on a SmartLab diffractometer (Rigaku Corporation, Japan) with Cu$K_{\alpha 1+\alpha 2}$ radiation (λ = 0.15419 nm). The lattice parameters were calculated using the PowderCell 2.4 software package (PowderCell for Windows, Version 2.4, 08.03.2000, Werner Kraus & Gert Nolze, BAM, Berlin). The microstructure of the samples was examined using a high-resolution scanning electron microscope (SEM) Quanta 3D FEG (Thermo Fisher FEI Company, USA) equipped with an additional FIB ion column and integrated EDAX Trident system.

Nanoindentation of the sample surface was performed using a Hysitron TI 950 TriboIndenter (Bruker, USA) equipped with a Berkovich indenter. Measurements were carried out along the sample diameter (70 ± 10 indents) at a constant loading rate of 40 mN/s. Before testing, the sample surfaces were polished with 1 μm diamond paste. The numerical values of nanohardness (H) and Young's modulus (E) were determined using the Oliver—Pharr method based on characteristic P—h curves [21—23]. Microhardness was measured with an ITV-1-MS tester (LLC Metotest, Russia) equipped with an MC-5.3 camera and LOMO-Microsystems MCview software. The average

microhardness values were obtained from ten indentations under a load of 100 g.

Human multipotent mesenchymal stromal cells (MSCs) obtained from the collection of the N.N. Blokhin National Medical Research Center of Oncology were used as a biological model. The cells were suspended in RPMI-1640 growt.h medium (PanEko, Russia) supplemented with 10 % fetal bovine serum, 1 % penicillin, and 2 mM L-glutamine (PanEko, Russia). The cell concentration was 132000 cells/mL.

The alloy samples were ultrasonically cleaned (Odaservice, Russia) in distilled water at 21 ± 1 °C for 15 min, immersed in 60 % ethanol for 4 h, air-dried under sterile conditions, and then placed individually in the wells of 24-well culture plates (Costar, USA). A 20 μL cell suspension was applied to each sample surface and incubated for 20 min at 37 °C in a 5 % CO_2 atmosphere. For control, an equal volume of cell suspension was placed directly on the well bottom. Then, 2 mL of growt.h medium was added to each well, and incubation continued for 1 and 5 days under the same conditions.

Biocompatibility of the studied alloy samples was assessed by comparing the viability of MSC cultures after 1 day of incubation using the MTT assay. The procedure was performed as described previously [22]. After 4 h of incubation, the supernatant was carefully removed, dimethyl sulfoxide was added to the cell sediment, and the optical density of the resulting solution was measured at 540 nm using a Spark microplate reader (Tecan, Switzerland) against the intact growt.h medium. The alloys were considered biocompatible if their exposure did not result in a statistically significant decrease in cell viability compared with the control.

To evaluate cell adhesion, alloy samples with attached MSCs were treated with Calcein AM solution (Sigma, USA) according to the manufacturer's protocol after 5 days of cultivation. Cells on the sample surfaces were examined using fluorescence microscopy on a LionHeart FX cell imaging system (Perkin Elmer, USA). For quantitative assessment of surface cell adhesion intensity, the alloy samples with adhered cells were transferred to empty wells, and growt.h medium was removed from the control wells. Then, 1 mL of fresh growt.h medium was added to both sample and control wells, and the viability of adhered cells was evaluated using the MTT assay as described above.

Statistical analysis was performed using at least three samples of each alloy type for every parameter. The results for MSC viability and cell adhesion were expressed as mean ± standard deviation based on triplicate measurements. Comparative analysis was carried out using the median criterion, and differences from the control were considered statistically significant at $p < 0.05$.

Results

Microstructure and phase composition of samples in the as-fabricated, annealed, and HPT-processed states

SEM and XRD results were obtained for both investigated alloys in three conditions: as-fabricated (after ingot production), after annealing, and after high-pressure torsion (HPT). The samples in the as-fabricated condition (Fig. 1, *a*, *b*) and after annealing (Fig. 1, *c*, *d*) exhibited coarse-grained polycrystalline structures. It should be noted that the distribution of alloying components was nonuniform (Fig. 1, *a*), and each grain consisted of subgrains with an average size of 100—150 nm.

Significant changes in the microstructure occurred after HPT processing (Fig. 1, *e*, *f*). The grain size could not be determined from the SEM images; however, other structural features, such as shear bands, were clearly visible. On the surface of the Ti—15Mo alloy (Fig. 1, *f*), these bands appear as separate regions, which most likely correspond to differently oriented grains.

Fig. 2 presents the *X*-ray diffraction patterns for both alloys in the three studied states.

Tables 1 and 2 summarize the XRD data, including the phase composition, lattice parameters, and volume fractions of phases in the Ti—10Mo and Ti—15Mo alloys. In all investigated samples, the main phase was the β-Ti phase.

Based on the *X*-ray diffraction analysis, the volume fraction of the β-Ti phase (Fig. 3, *a*) and its lattice parameters (Fig. 3, *b*) were calculated for both alloys in all three conditions. In the as-fabricated and annealed conditions, the samples exhibited coarse-grained structures, and the volume fraction of the β-Ti phase was nearly identical. The lattice parameters of the annealed samples differed significantly from those in the as-fabricated condition due to the enrichment of the β-Ti phase with molybdenum. In the Ti—10wt.%Mo alloy, all molybdenum atoms are incorporated into the β-Ti phase, whereas in the Ti—15wt.%Mo alloy, the excess molybdenum forms a secondary cubic β-Mo phase.

Phase transformations induced by HPT processing altered not only the β-Ti/β-Mo phase ratio but also the

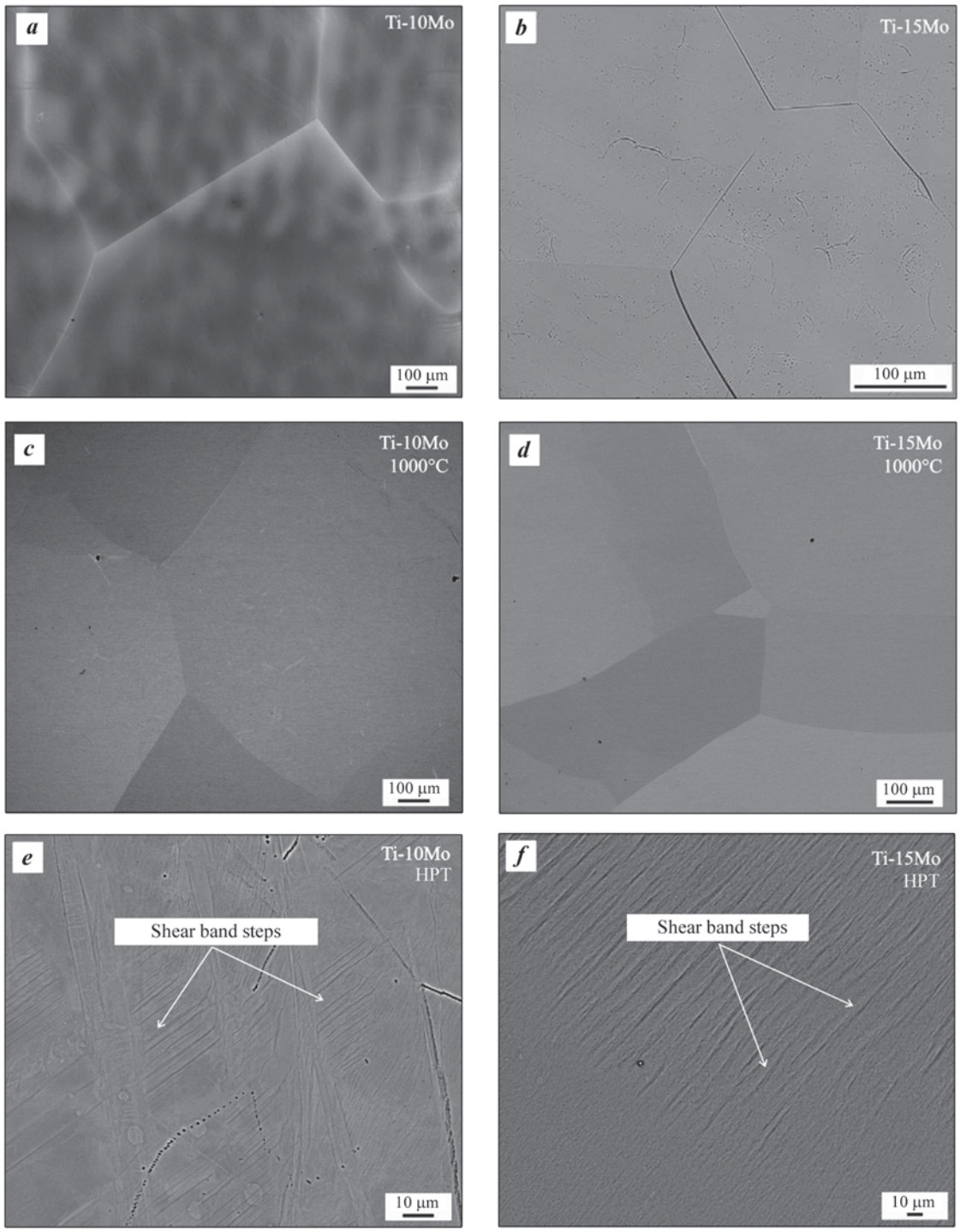

Fig. 1. SEM micrographs of the alloy microstructures

a, ***b*** – as-fabricated; ***c***, ***d*** – after annealing; ***e***, ***f*** – after high-pressure torsion (HPT) processing
a, ***c***, ***e*** – Ti–10Mo alloy; ***b***, ***d***, ***f*** – Ti–15Mo alloy

Рис. 1. СЭМ-изображения микроструктур сплавов

a, ***b*** – в исходном состоянии; ***c***, ***d*** – после отжига, ***e***, ***f*** – после КВД-обработки
a, ***c***, ***e*** – сплав Ti–10Mo; ***b***, ***d***, ***f*** – сплав Ti–15Mo

Table 1. **Phase composition, lattice parameters, and phase fraction for the Ti–10Mo alloy**

Таблица 1. Фазовый состав, параметры решеток и доля фаз для сплава Ti–10Mo

Alloy condition	α			βTi		βMo	
	V, %	*a*, nm	*c*, nm	*V*, %	*a*, nm	*V*, %	*a*, nm
As-fabricated	4	0.2951	0.4688	84	0.3257	12	0.3216
After annealing (1000 °C)	–	–	–	100	0.3275	–	–
After HPT	–	–	–	99.5	0.3259	–	–

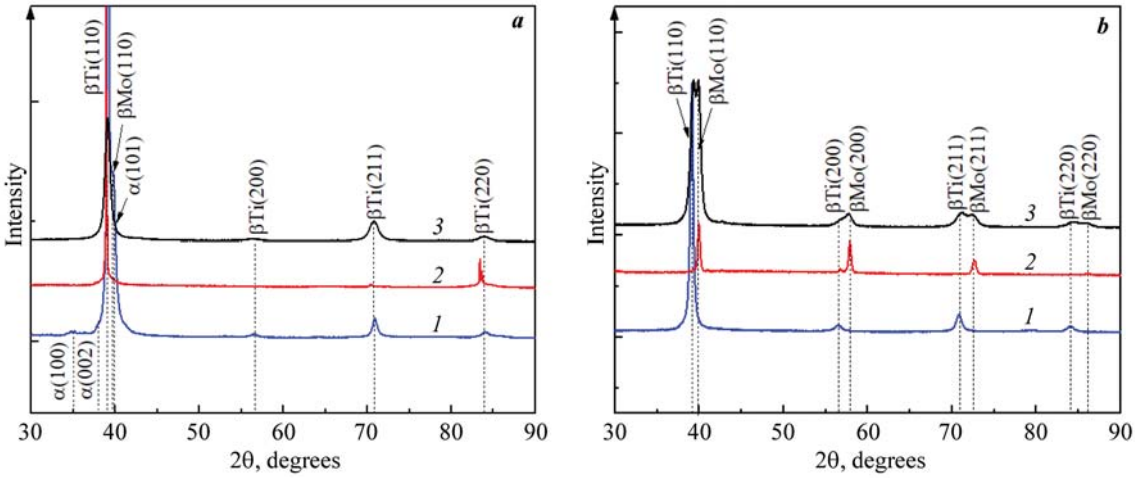

Fig. 2. *X*-ray diffraction patterns of Ti–10Mo and Ti–15Mo alloys in three conditions: as-fabricated (***1***), annealed at $t = 1000$ °C (***2***), and HPT-processed (***3***)
a – Ti–10Mo, ***b*** – Ti–15Mo

Рис. 2. Рентгенограммы образцов сплавов Ti–10Mo и Ti–15Mo в исходном состоянии (***1***), после отжига при $t = 1000$ °C (***2***) и после КВД-обработки (***3***)
a – Ti–10Mo, ***b*** – Ti–15Mo

Table 2. **Phase composition, lattice parameters, and phase fraction for the Ti–15Mo alloy**

Таблица 2. Фазовый состав, параметры решеток и доля фаз для сплава Ti–15Mo

Alloy ondition	βTi		βMo	
	V, %	*a*, nm	*V*, %	*a*, nm
As-fabricated	100	0.3257	–	–
After annealing (1000 °C)	94	0.3246	4	0.3189
After HPT	55	0.3246	45	0.3196

corresponding lattice parameters of the solid-solution phases.

Mechanical properties of the alloys

The microhardness of the studied samples was measured (Fig. 4, *a*), while nanoindentation was used to determine the average values of nanohardness (Fig. 4, *b*) and Young's modulus (Fig. 4, *c*). The dependences of microhardness and nanohardness exhibited similar trends for both alloys. An increase in molybdenum content in the titanium alloy resulted in lower values of these parameters. The microstructure, phase composition, and mechanical properties of the as-fabricated and annealed samples were almost identical. Essentially, annealing at

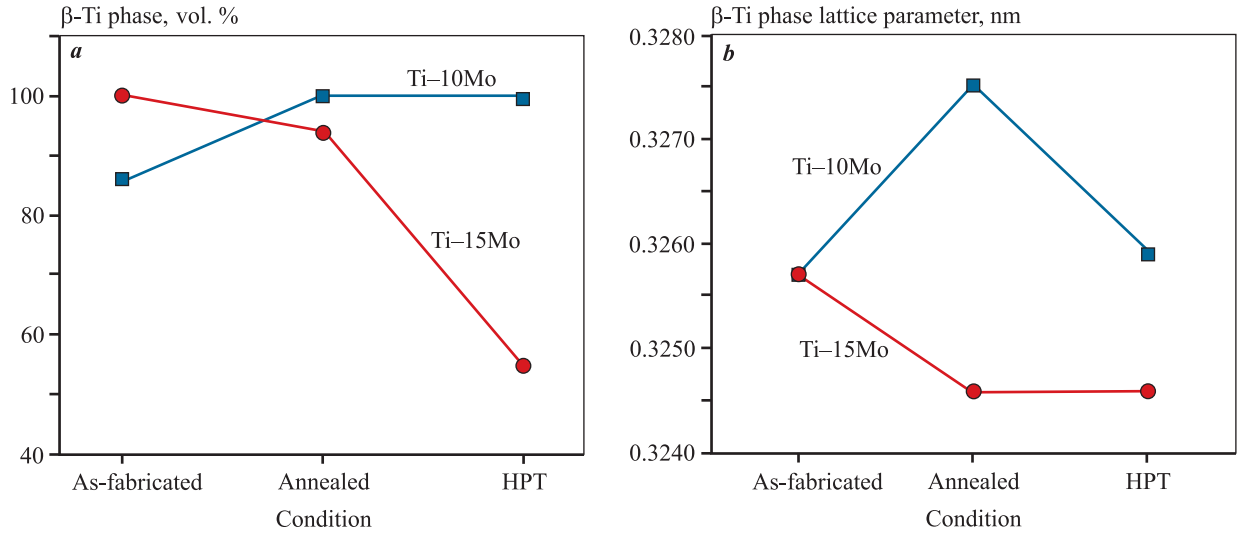

Fig. 3. Volume fraction of the β-Ti phase (***a***) and lattice parameter of the β-Ti phase (***b***) for Ti–10Mo and Ti–15Mo alloys depending on the processing method

Рис. 3. Зависимости объемной доли βTi-фазы (***a***) и параметров решетки βTi-фазы (***b***) для сплавов Ti–10Mo и Ti–15Mo от вида обработки

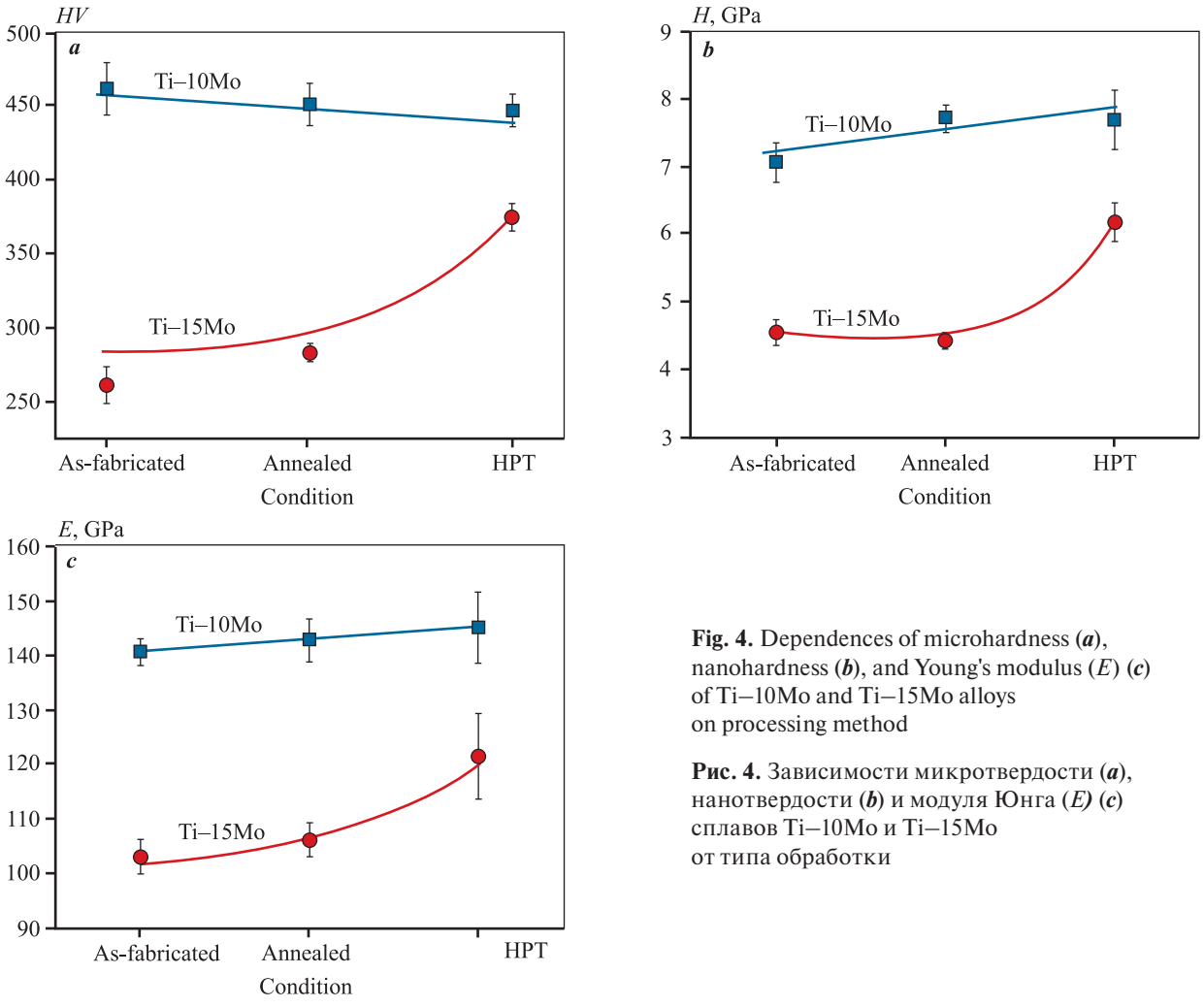

Fig. 4. Dependences of microhardness (***a***), nanohardness (***b***), and Young's modulus (*E*) (***c***) of Ti–10Mo and Ti–15Mo alloys on processing method

Рис. 4. Зависимости микротвердости (***a***), нанотвердости (***b***) и модуля Юнга (*E*) (***c***) сплавов Ti–10Mo и Ti–15Mo от типа обработки

1000 °C served as a homogenization treatment that eliminated chemical and structural inhomogeneities in the samples. It is well known that severe plastic deformation refines the grain size and induces phase transformations in metals and alloys, which consequently alters their mechanical properties. For the Ti—10 Mo alloy, such changes were not observed. It can be assumed that the key factor influencing the mechanical properties of the investigated alloys was the phase transformation induced by HPT processing, specifically the formation of the β-Mo phase in the Ti—15 Mo alloy, which led to changes in the mechanical behavior.

Biocompatibility of the samples and stimulation of cell adhesion

Human multipotent mesenchymal stromal cells (MSCs) were used as a cellular model because, depending on external stimuli, they are capable of differentiating into various connective tissue lineages involved in the formation of bone, cartilage, stroma, muscle, blood vessels, and tendons [23]. These are precisely the types of tissues expected to interact with the developed implants following their placement in osteoreconstructive surgery. To ensure stable osteosynthesis, the metallic implant material must comply with biocompatibility requirements — namely, it should not exert cytotoxic effects upon contact and should promote surface cell adhesion.

Morphologically, MSCs are large polygonal cells measuring approximately 20—50 μm in diameter. They adhere effectively to the matrix surface, maintain viability and proliferative capacity *in vitro*, and are capable of colonizing the surfaces of experimental alloy samples (Fig. 5). Live-cell staining with Calcein AM enables visualization of metabolically active cells through the fluorescence emitted by the cytoplasm of viable cells.

This methodological approach confirmed that the cells adhered to the surfaces of all studied Ti—Mo alloy samples (Fig. 6). The analysis showed that the cells not only adhered actively during co-incubation but also retained their ability to proliferate, forming continuous cell layers on the alloy surfaces. Overall, the degree of cell colonization was comparable among the samples, though cell activity on the surface of the Ti—15Mo alloy was slightly higher than on Ti—10Mo. In contrast, the colonization of the Ti—10Mo surface after HPT processing was somewhat reduced compared to that of the as-fabricated and annealed samples.

The data presented in Fig. 7, *a* indicate that none of the studied samples exhibited statistically significant cytotoxic effects on MSCs. After 24 h of incubation, no

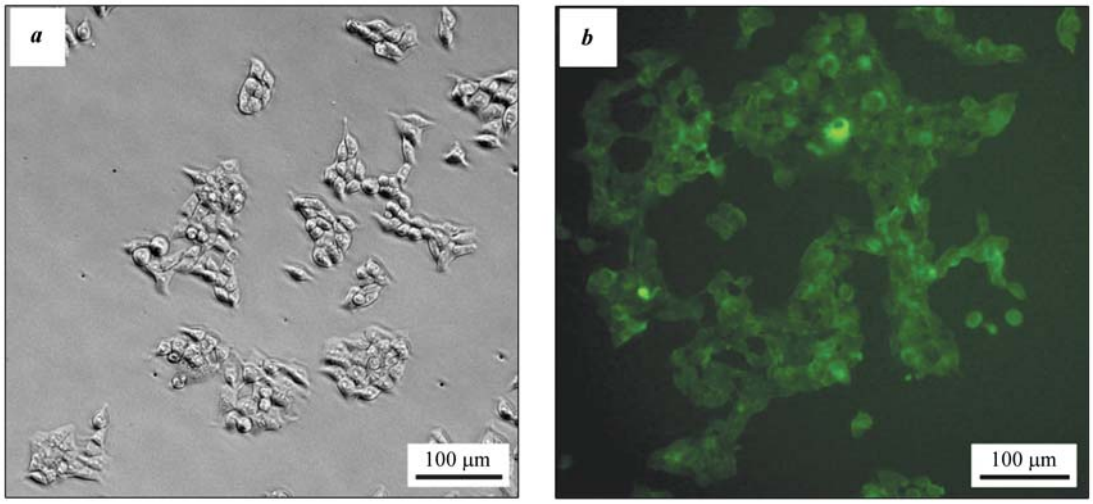

Fig. 5. Morphology, viability, and adhesion potential of MSCs (control) used as a cellular model for evaluating the adhesion properties of the studied alloys

a – phase contrast microscopy, unstained
b – fluorescence microscopy, with phase contrast Calcein AM cell staining

Рис. 5. Морфология, жизнеспособность и адгезионный потенциал ММСК (контроль), использованных в качестве клеточной модели для оценки адгезионных свойств изучаемых сплавов

a – фазово-контрастная микроскопия, без окраски
b – флуоресцентная микроскопия, дополненная использованием фазового контраста, окраска клеток «Calcein AM»

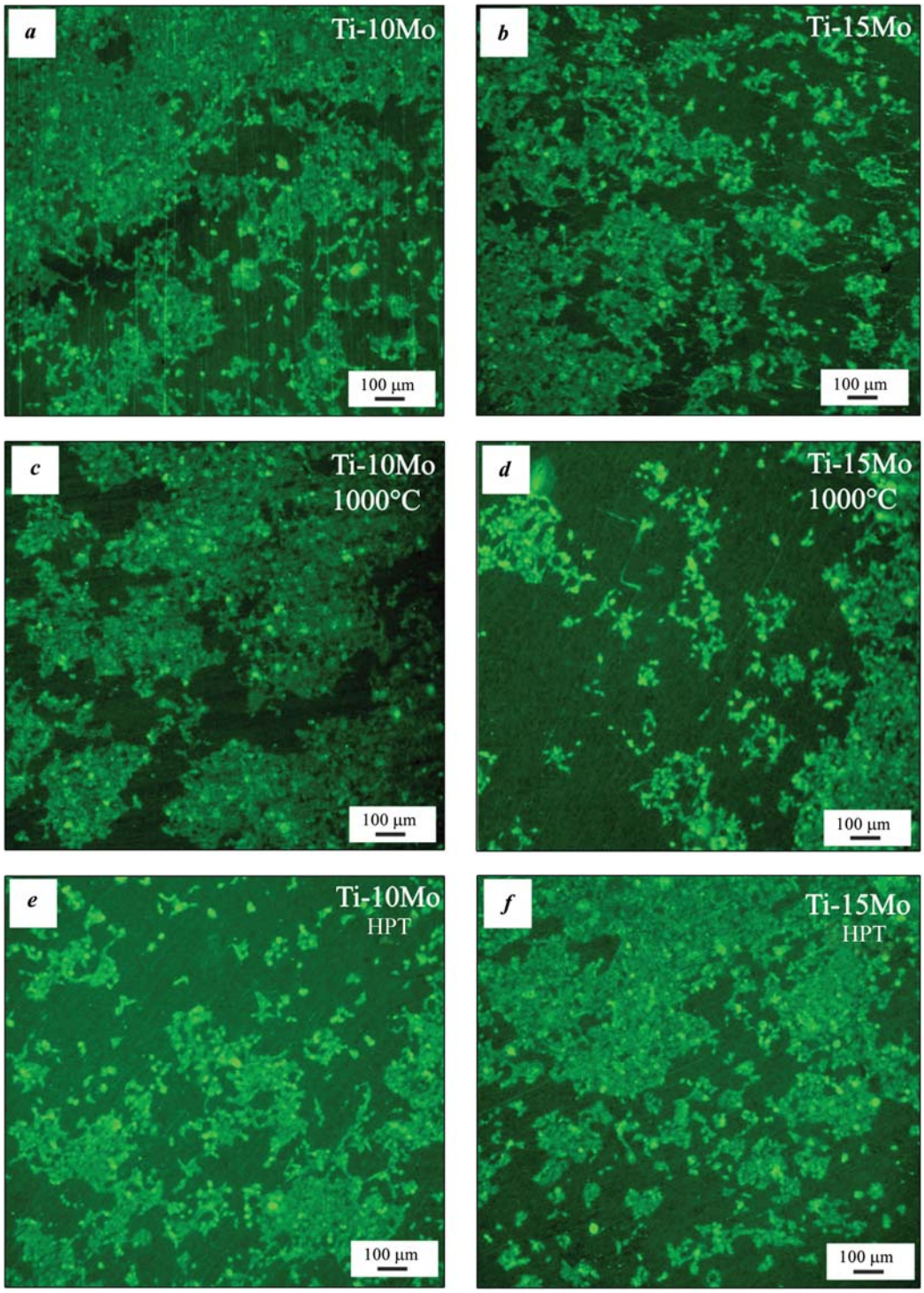

Fig. 6. Colonization and adhesion of MSCs on the surface of Ti–Mo alloy samples

Fluorescence microscopy, Calcein AM staining, green fluorescence
a, ***b*** – as-fabricated; ***c***, ***d*** – after annealing (1000 °C); ***e***, ***f*** – after HPT processing
a, ***c***, ***e*** – Ti–10 Mo alloy; ***b***, ***d***, ***f*** – Ti–15 Mo alloy

Рис. 6. Колонизация и адгезия ММСК на поверхности образцов сплавов на основе Ti–Mo

Флуоресцентная микроскопия. Окраска клеток – «Calcein AM» (зеленая флуоресценция)
a, ***b*** – в исходном состоянии; ***c***, ***d*** – после отжига (1000 °C), ***e***, ***f*** – после КВД-обработки
a, ***c***, ***e*** – сплав Ti–10Mo; ***b***, ***d***, ***f*** – сплав Ti–15Mo

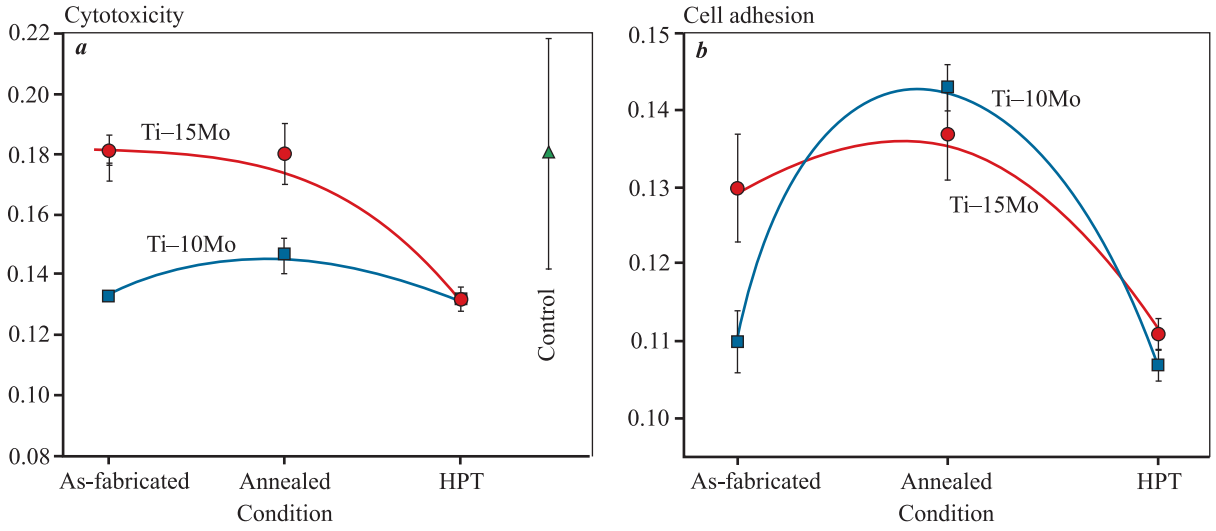

Fig. 7. Dependences of cytotoxicity (***a***) and cell adhesion (***b***) of Ti–10wt.%Mo and Ti–15wt.%Mo alloys on processing method

Рис. 7. Зависимости цитотоксичности (***a***) и клеточной адгезии (***b***) сплавов Ti–10Mo и Ti–15Mo от вида обработки

decrease in cell viability was observed compared to the control ($p > 0.05$), which meets the requirements for biocompatible materials intended for medical applications. However, HPT processing appeared to induce a slight trend toward reduced viability.

Quantitative assessment of cell activity on the alloy surfaces at the end of the observation period demonstrated that the highest activity was observed in annealed samples, while the lowest values corresponded to the HPT-processed samples.

Discussion of the results

Differences in fabrication methods and in the purity of the starting components affect not only the phase composition of the ingots, but also, consequently, the mechanical properties of the alloys. For example, in [5] a series of binary Ti—Mo alloys containing 6—20 wt. % Mo was studied. The ingots were produced by industrial arc melting, and the alloys were examined in the as-cast state without any additional processing. Two alloys — Ti—10wt.%Mo and Ti—15wt.%Mo — consisting solely of the β phase exhibited different mechanical properties. In particular, the bending strength and hardness of the alloy containing 10 wt. % Mo were higher than those of Ti—15wt.%Mo. However, when these data are compared with the results of the present study, differences in the phase composition of the same nominal alloys become apparent, which in turn lead to discrepancies in their mechanical properties.

In [6], Ti—10wt.% Mo and Ti—20wt.%Mo alloys were investigated, and the authors demonstrated that both the mechanical properties and the microstructure are strongly dependent on the processing method (in that case, cold rolling). They concluded that the Ti—Mo alloys under study are more suitable for biomedical applications than conventional metallic biomaterials, as they combine a low yield strength with good ductility, and highlighted Ti—10wt.%Mo as the most promising composition. Although a direct comparison between [6] and the present work is not feasible because of differences in processing and material characteristics, the results of [6] clearly support the view that the processing method has a decisive influence on both the phase composition and the properties of Ti—Mo alloys.

In [7], a series of binary Ti—Mo alloys with 15—18 wt. % Mo was examined. The Ti—17wt.% Mo alloy exhibited low elasticity, high tensile strength, and good ductility, making it a promising candidate for biomedical use.

The Ti—15.05wt.%Mo alloy developed by the group of authors in [8] also demonstrated good potential for orthopedic implant applications, with a hardness of 350 HV and an elastic modulus of 70 GPa.

It is also worth mentioning the study [9], in which nanoindentation was used to measure the hardness and Young's modulus of individual phases. For the

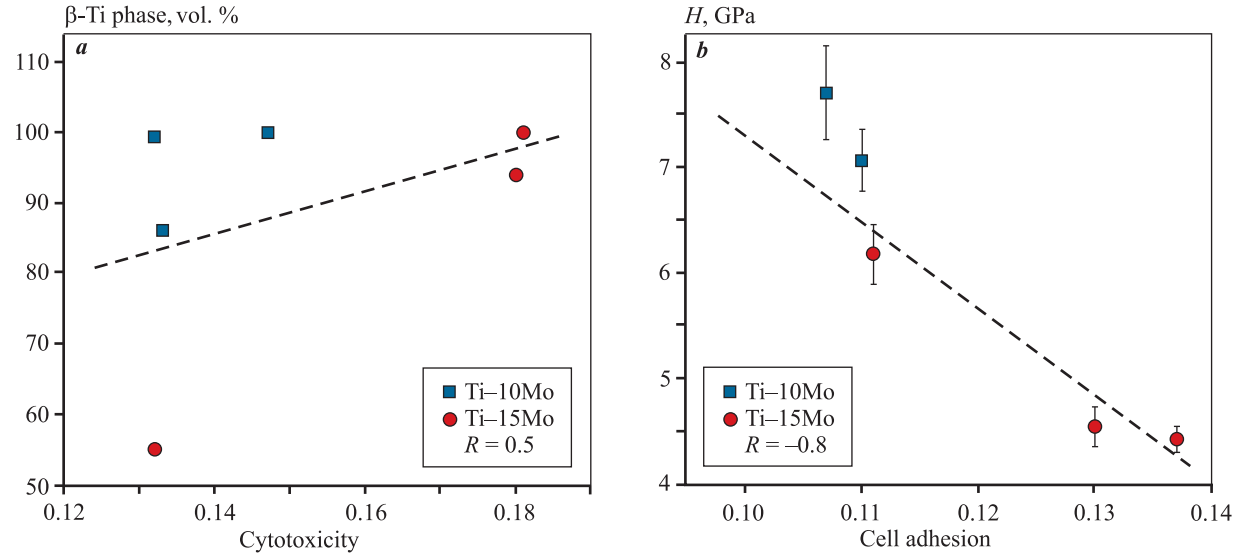

Fig. 8. Dependences of cytotoxicity (***a***) and cell adhesion (***b***) of Ti–10Mo and Ti–15Mo alloys on the volume fraction (***a***) of the β-Ti phase and hardness (***b***)

Рис. 8. Зависимости цитотоксичности (***a***) и клеточной адгезии (***b***) сплавов Ti–10Mo и Ti–15Mo от доли βTi-фазы (***a***) и твердости (***b***)

Ti—12Mo alloy, the difference in hardness between the α and β phases was approximately 35 % (3.96 and 5.97 GPa, respectively). The difference in Young's modulus between the two phases was about 20 % (114 GPa for the β phase and 141.7 GPa for the α phase). The alloys in [9] were produced by spark plasma sintering (SPS) from titanium and molybdenum powders.

Based on current evidence regarding the impact of fabrication and processing on material properties, we focused this study on two alloys, Ti—10wt.%Mo and Ti—15wt.%Mo, which exhibit mechanical characteristics suitable for biomedical use. The present work provides a comprehensive characterization of these alloys, including SEM and XRD analysis, nanoindentation, and biocompatibility assessment.

Evaluation of cell viability during incubation showed that all studied samples can be classified as biocompatible according to this parameter, although the annealed samples were found to be the safest for MSC viability. According to the results of cell adhesion testing, all Ti—Mo alloy samples stimulated MSC adhesion to their surfaces, with the annealed samples exhibiting the most pronounced activity. Considering the osteogenic potential of MSCs, it can be concluded that the Ti—10Mo and Ti—15Mo alloys, after heat treatment at 1000 °C, can be characterized as biocompatible materials with osteoconductive potential, i.e., capable of promoting bone tissue formation on their surface to accelerate implant osseointegration.

A possible correlation between cytotoxicity and cell adhesion and such material parameters as phase composition, hardness, and Young's modulus was analyzed. A positive correlation coefficient ($R = 0.5$) was obtained for the volume fraction of the β-Ti phase with both cytotoxicity (Fig. 8, *a*) and cell adhesion. Negative correlation coefficients (R) were found for hardness (Fig. 8, *b*) and Young's modulus.

Based on the correlation coefficient analysis, it can be proposed that the key factor determining the biocompatibility of Ti—Mo alloys is not their mechanical properties, but rather the phase composition and the type of processing applied to the material.

Conclusion

The experiments performed in this study demonstrated that all investigated Ti—Mo alloy samples can be classified as biocompatible, since, during *in vitro* incubation with non-transformed cells, they did not exert any statistically significant cytotoxic or hemolytic effects. In addition, all samples were found to stimulate surface colonization by cells with osteogenic potential, which suggests that, following intrabony implantation, they may undergo accelerated integration into bone tissue by promoting callus formation in the contact area.

It was shown that HPT processing of the materials reduces both cytotoxicity and cell adhesion parameters

for both Ti—Mo alloys. At the same time, higher levels of cell adhesion and cytotoxicity were recorded for the annealed Ti—Mo alloy samples.

The positive correlation coefficients calculated for cytotoxicity and cell adhesion as a function of the β-Ti phase fraction in both Ti—10wt.%Mo and Ti—15wt.%Mo alloys indicate the predominant influence of phase composition on the biocompatibility of the material.

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