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Original article

Assessment of the Biological Safety of Metal Alloys TNT (Ti21Nb6Ta) and BT-6 (Ti-6Al-4V)

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Abstract

The introduction of modern technologies in traumatology and orthopedics, such as immersion fixators and new biometallic alloys, has led to a qualitative change in approaches to treatment, but the use of new materials in the manufacture of implants remains relevant.

Purpose of the study. *Study of the biocompatibility of an alloy of titanium, tantalum and niobium (Ti21Nb6Ta) in vitro.*

Methods*. The study examined the toxic effects of aqueous extracts of TNT (Ti21Nb6Ta) and BT-6 (Ti-6Al-4V) on human fibroblasts and periosteal cells in vitro using the MTT test; hemolytic activity of TNT and BT-6 alloys on the blood of experimental animals - Wistar rats (in vitro) by spectrophotometry; Pyrogenic activity was also studied using the kinetic chromogenic method. The object of the study is the metal alloys TNT (Ti21Nb6Ta) and BT-6 (Ti-6Al-4V) (control group).*

Results*. In vitro studies have shown that aqueous extracts from the metal alloys TNT and BT-6 do not have a cytotoxic effect on cultured human fibroblasts and periosteal cells in vitro. It was revealed that aqueous extracts of TNT and BT-6 alloys do not have hemolytic and pyrogenic activity.*

Conclusions. *The studies conducted indicate a high level of safety and biocompatibility of the TNT alloy, which is proven by a set of in vitro tests. Thus, according to the generally accepted hygienic classification, the metal alloys TNT (Ti21Nb6Ta) and BT-6 (Ti-6Al-4V) can be classified as hazard class 4 - low-hazard substances (GOST 12.1.007-76).*

Key words: *metal alloy, Ti-Nb-Ta, toxicity, biocompatibility, hemolysis, pyrogenicity.*

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Introduction

The high level of trauma and diseases of the musculoskeletal system in the Republic of Kazakhstan requires the optimization of specialized medical institutions, including structural changes and equipment updates. The implementation of modern osteosynthesis systems has led to significant changes in the methods of treatment in the field of traumatology and orthopedics, including the use of advanced methods, tools, and materials.

The promotion of surgical treatment methods in traumatology and orthopedics is closely linked to the integration of new technological innovations into the production process of implants, which are an integral part of surgical practice [1,2]. However, today, the main properties of implants are clinical effectiveness, device strength, universality, and economic viability. The possibility of early patient mobilization after surgical intervention, minimally invasive procedures, and a reduction in the risk of complications have led to an increased use of intramedullary fixators, as opposed to the late 20th century when external fixation devices predominated [3,4].

The introduction of new alloys, composite materials, and various types of coatings has clearly improved the effectiveness of osteosynthesis [5]. Despite significant advancements in the development of implants for traumatology and orthopedics, there remains a relatively high level of risks and complications [6,7]. On one hand, this may be associated with errors in the choice of treatment method, surgical technique, and the presence of concomitant pathology in patients, including allergic reactions to metal. On the other hand, there are still issues with the quality of alloys used in osteosynthesis structures [8,9,10].

It is known that the body as a whole and the tissues surrounding the implant can pathologically react to a foreign agent. In particular, metallic implants do not always remain inert in the aggressive environment of biological fluids [11,12]. Unfortunately, the problem persists due to the use of low-quality materials in the manufacture of implants. The term "metallosis" is still used to describe complications after osteosynthesis and infections around implants remain a serious problem in surgery [13,14].

Surgical implants are usually made from metallic materials, including austenitic stainless steel, cobalt-chromium alloys, as well as titanium and its alloys [15]. In the 1940s, titanium began to be used as a material for medical implants due to its favorable properties, including low density, exceptional mechanical characteristics, and lack of toxicity [16]. It is known that metal ions can be released from metallic materials due to corrosion. There are studies showing that metal ions formed as a result of corrosion of austenitic stainless steels in vitro can lead to changes in the expression of human lymphocyte surface antigens and hinder the immune response, as evidenced by a reduction in lymphocyte proliferation [15,17,18]. Local adverse tissue or allergic reactions associated with metallic implants result from the release of ions from these metals. The degree of ion release depends on the corrosion rate of the alloy and the solubility of the initial corrosion by-products [19]. Nevertheless, the interaction of implants with body fluids over an extended period inevitably leads to corrosion, thereby exerting a toxic effect on surrounding tissues and the organism as a whole.

The aim of the study is to conduct preclinical research to assess the safety and biocompatibility of the titanium, tantalum, and niobium alloy TNT (Ti21Nb6Ta) under in vitro conditions.

Materials and methods

The study of the new alloy TNT (Ti21Nb6Ta) was conducted over a period of 4 months, examining various interactions of the TNT alloy (Ti21Nb6Ta) with living cells of the organism (in vitro) and directly within a living organism (in vivo). The research presented in this article was carried out at the National Center for Biotechnology in Astana.

The study design was an experimental preclinical study, consisting of two stages: the first stage involved in vitro tests, and the second stage comprised in vivo tests to gain a more comprehensive understanding of the safety, biocompatibility, and osseointegration of the tested alloy.

The results of the first stage, focusing on toxic effects of aqueous extracts from TNT (Ti21Nb6Ta) and BT-6 (Ti-6Al-4V) (control group) on primary cultures of human dermal fibroblasts (HDFn) and human periosteum cells under in vitro conditions, were documented in the article. This was done using the MTT test. Additionally, hemolytic activity of TNT and BT-6 alloys on the blood of experimental animals (Wistar rats) was assessed in vitro using spectrophotometry. The pyrogenic activity of the studied alloy was also investigated by Chromogenic Limulus Amebocyte Lysate Test (LAL test). The study focused on metal alloys TNT (Ti21Nb6Ta) and BT-6 (Ti-6Al-4V) as the control group.

Ethical Issues. The work plan for studying the acute toxicity and irritant action of metal alloys TNT and BT-6 was developed in accordance with the guidelines outlined in the manual for conducting preclinical studies of medicinal products and medical devices [20], following the principles of the Helsinki Declaration of the World Medical Association (revised in 2013) [21]. Animal research was conducted after obtaining approval from the Local Ethics Committee of the National Center for Biotechnology on September 23, 2019.

Metrological support of the research. All measurement instruments and test equipment used in the scientific studies underwent verification and certification procedures in the relevant accredited bodies — Akmolinsky branch of the National Center for Expertise and Certification and the RSE "Kazakhstan Institute of Metrology."

The object of the study was the experimental alloy TNT (Ti21Nb6Ta), as well as samples of the titanium alloy BT-6 (Ti-6Al-4V) – the control group provided by the Ulbinsky Metallurgical Plant.

Cell Lines. Primary cultures of fibroblasts (HDFn) from human skin and human periosteum cells were used to determine the general toxic effects of extracts from the alloys. HDFn cell cultures and human periosteum cells were obtained from the RSE "National Center for Biotechnology" of the Ministry of Education and Science of the Republic of Kazakhstan. The use of HDFn fibroblast cells as a primary cell model, despite their primary cell status, is convenient due to their easy accessibility and adaptability to cell culture. Fibroblasts (HDFn) can be used to at least double the population by a factor of 20, which is sufficient for many tests [22]. The use of human periosteum cells is justified by the direct contact of metal with the periosteum during osteosynthesis, making them a representative model for testing the safety of implanted products in traumatology and orthopedics.

Preparation of Aqueous Extracts from Metal Alloy Samples TNT and BT-6. The preparation of aqueous extracts from metal alloy samples TNT and BT-6 was carried out in accordance with GOST ISO 10993-12-2011 (Medical devices. Evaluation of the biological action of medical devices. Part 12. Preparation of samples and control samples) [23]. Aqueous extracts of metal alloys TNT and BT-6 were sterilized by autoclaving at 121°C for 30 minutes. Then, the samples were placed in a sterile tube with a 50 ml solution of 0.9% sterile NaCl. The sample-to-solution ratio was 1g:10ml. Subsequently, the tube with the samples was incubated in a thermostat at 37°C for 24 hours. Afterward, the aqueous extracts were stored in the refrigerator at +4°C for temporary storage.

Evaluation of the general toxicity of TNT and BT-6 on human cell culture. Neonatal human skin fibroblasts (HDFn) and human periosteum cells were dispersed in wells of a 96-well plate (BD Biosciences, USA) at a density of 1×104 cells/well. The cells were incubated in Dulbecco's Modified Eagle Medium (DMEM) with high glucose content, containing 10% fetal bovine serum (FBS), overnight at 37°C and 5% CO2. Subsequently, the culture medium was removed from the wells using a pipette, and 100 µl of the tested TNT and BT-6 samples at various dilutions were added. DMEM with 10% FBS served as the control. The cells were then incubated for 24 hours at 37°C and 5% CO2.

After 24 hours of incubation, the culture medium in each well of the 96-well plate was replaced with 90 µl of fresh phenol red-free culture medium. Subsequently, 10 µl of the MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (5 mg/ml) was added to each well. The cells were incubated for 4 hours at 5% CO2 and 37°C. After incubation, the medium was aspirated, and the formazan was dissolved by adding 100 µl of dimethyl sulfoxide (DMSO) per well. Optical density was recorded using a BioRad 680 plate spectrophotometer (Biorad, France) at a wavelength of 580 nm. MTT test data were obtained by comparing optical density (OD) in experimental groups.

The optical density is proportional to the number of viable cells in the well. Changes in OD were used to assess the cytotoxic activity of the tested samples. Cell viability was calculated using the formula: Atest/Acontrol*100, where Atest is the optical density of the test wells, Acontrol is the optical density of the control wells, and 100 represents 100% viability.

Evaluation of Hemolytic Activity of Metal Alloys TNT and BT-6. The hemolytic test is a widely used method for assessing the hemocompatibility of biomaterials, measuring the degree of erythrocyte disruption and hemoglobin release. This test is more sensitive to toxic substances than reactions of other cells in the body. A lower hemolytic coefficient indicates better hemocompatibility [24, 25].

The hemolytic activity of aqueous extracts of metals was studied by exposing isolated erythrocytes from three Wistar rats to the extracts in vitro. It was considered that, under normal conditions, the percentage of lysed cells should not exceed 2%. The principle of the method involves determining the hemolytic action of the extract by 100% hemolysis of erythrocytes.

Procedure for preparing the metal extract: Samples of TNT and BT-6 alloys (1.5 g each) were placed in a glass flask with a ground stopper and filled with 50 ml of a 0.9% aqueous solution of sodium chloride. The flask was placed in a thermostat and kept for 14 days at a temperature of 37-40°C. The resulting solution was used to study the toxic properties of the alloys.

Preparation of erythrocyte mass from the blood obtained from three rats was carried out as follows: blood was drawn from the carotid artery, adding 0.5 ml of a 3.9% solution of sodium citrate to each syringe before drawing blood to prevent coagulation. Then, blood was drawn in an amount of 4.5 ml. The ratio of 0.15 ml:1.5 ml (1:9) of sodium citrate solution to blood, respectively, was strictly observed. The erythrocyte mass prepared in this way can be stored for up to 72 hours at a temperature of 4-6°C. In the next stage, washing of erythrocytes from the destroyed elements of blood was performed. The erythrocyte mass was poured into three test tubes, each containing 5 ml, and centrifuged for 10 minutes at 2000 rpm. The supernatant was poured off, and 9 ml of sterile 0.9% sodium chloride solution was added to the sediment. The contents were mixed, and centrifugation was repeated for another 10 minutes at 2000 rpm. Visual assessment of the transparency of the supernatant was carried out. It was poured off, 9 ml of sterile sodium chloride solution was added, and the same centrifugation procedure was repeated. After centrifugation and visual assessment of transparency, another cycle of washing erythrocytes was required: pouring off the cloudy pink supernatant, adding 9 ml of 0.9% sodium chloride solution, and centrifugation. Visual assessment of transparency yielded a positive result: a clearly separated sediment - intact blood elements (erythrocytes); the supernatant - colorless, transparent, without signs of hemolysis.

Preparation of a 10% suspension of erythrocytes. From the bottom of each test tube after centrifugation, the sediment (formed elements) was taken in an amount of 1 ml and mixed with 9 ml of a 0.9% NaCl solution. Three variants (three test tubes) of erythrocyte suspensions were obtained. Such a mixture is allowed to be stored for no more than 24 hours at a temperature of 4-6°C. Next, the preparation of control samples and samples with 100% hemolysis followed. Nine test tubes were used, three lines of three test tubes for each rat: experimental, control, 100% hemolysis. In the experimental test tubes, 5 ml of the alloy extract with previously added 0.9% NaCl were placed. In the control test tubes, 5 ml of 0.9% NaCl solution was added. In the test tubes with 100% hemolysis, 5 ml of distilled water was added. Then, using a graduated measuring pipette, 0.5 ml of a 10% suspension of erythrocytes was added to each test tube. In the test tubes with 100% hemolysis, the formation of "lacquer blood" (100% hemolysis) was immediately observed due to the difference in osmotic pressure between distilled water and a 10% suspension of erythrocytes. The test tubes were thermostated for 1 hour at a temperature of 37°C, then centrifuged for 20 minutes at 2000 rpm. After centrifugation, the supernatant was separated for optical density measurements. The optical density of the experimental, control, and 100% hemolysis samples was measured on a Bio-Rad 680 spectrophotometer at a wavelength of 540 nm against a "blank" sample (water).

Evaluation of the pyrogenicity of metal alloys TNT and BT-6. The chromogenic Limulus Amebocyte Lysate Test (LAL test) is based on the formation of a colored product during the hydrolysis of a substrate, which occurs only when the enzyme system is activated in the presence of bacterial endotoxin (BE). In the presence of lipopolysaccharides, the tested solutions acquire a yellow color, with the color intensity directly corresponding to the concentration of BE.

This feature allows for the construction of a calibration curve and facilitates the quantitative assessment of BE levels. The peak sensitivity of the method is 0.005 endotoxin units per milliliter (EU/ml) [26].

To assess the pyrogenicity of alloys, a kinetic chromogenic method using the LAL test was applied, as described in the European Pharmacopoeia (chapter 2.6.14) and the U.S. Pharmacopoeia (chapter 85). The pharmacopoeias refer to the KQCL method as method D. Measurement of endotoxin levels in samples was performed using the ENDOSAFE PTS endotoxin detection instrument (Charles River, USA). The chromogenic LAL test is based on the formation of a colored product resulting from the hydrolysis of a substrate, which occurs only when the enzyme system is activated in the presence of bacterial endotoxin. A notable advantage of the chromogenic LAL test is its ability to accurately determine the amount of BE and significantly improve method reproducibility [26].

Aqueous extracts of metal alloys TNT and BT-6 were used as the experimental and control groups, respectively.

Statistical processing of results was carried out using Microsoft Excel and Statistica 7. Distributions were described by the mean and standard error of the mean. Intergroup differences were evaluated by the non-parametric Mann-Whitney U-test. Differences were considered significant at a 95% probability threshold ($p<0.05$).

Results

According to the results of the study on the acute toxicity of aqueous extracts of TNT and BT-6 alloys on human periosteal cells and fibroblasts, based on Figures 1 and 2, the highest level of cell viability was observed in the 0.9% NaCl solution (false control) – 100 and 94.12%; BT-6 (control) – 82.6 and 87.5%; TNT (main group) – 66.25 and 72.88%, respectively.

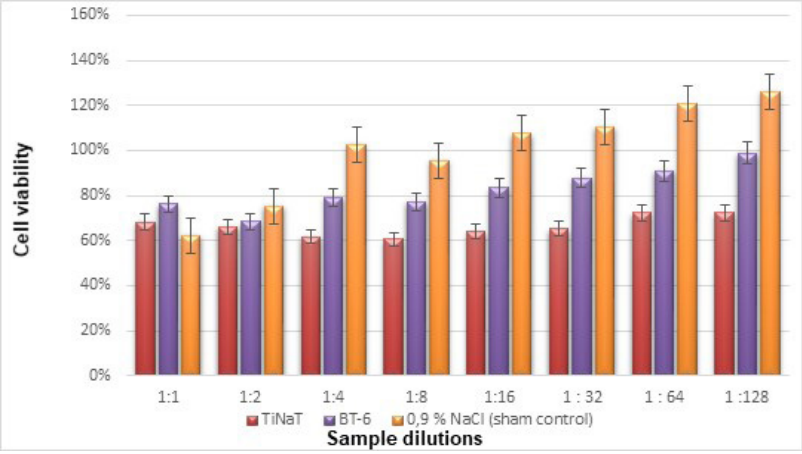

Figure 1 - Viability of human periosteal cells after 24 hours of incubation with samples of aqueous extracts of metal alloys TNT (Ti21Nb6Ta) and BT-6 (Ti-6Al-4V)

The obtained p-value is 0.21, and at a 95% probability threshold ($p<0.05$), there were no statistically significant differences between the groups (Figures 1 and 2).

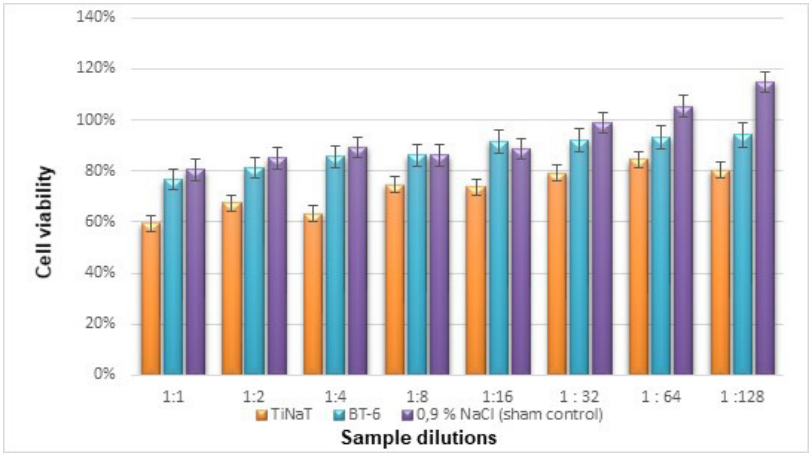

Figure 2 - Viability of human skin fibroblasts HDFn after 24 hours of incubation with samples of aqueous extracts of metal alloys TNT (Ti21Nb6Ta) and BT-6 (Ti-6Al-4V)

The examination of toxic properties revealed that aqueous extracts from TNT and BT-6 metals do not possess in vitro hemolytic activity when acting on isolated rat erythrocytes (hemolysis was absent) within permissible values of not more than 2% (Figure 3).

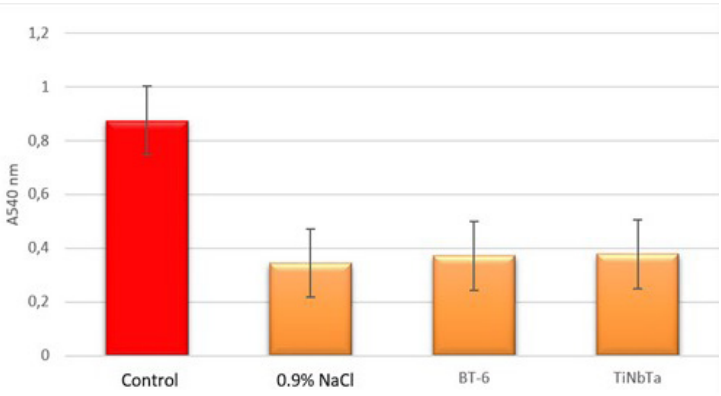

Figure 3 - Hemolytic activity of metal alloys TNT (Ti21Nb6Ta) and BT-6 (Ti-6Al-4V)

The results of the LAL test showed that the investigated aqueous extracts of metal alloys TNT and BT-6 are non-pyrogenic, as the level of bacterial endotoxins was 0.05 EU/ml, which corresponds to the absence of pyrogenic properties in TNT and BT-6 alloys.

Discussion

In our study, composite alloys TNT (Ti21Nb6Ta) were used due to the excellent biomechanical properties of each element in the composite alloy. Metallic biomaterials, such as tantalum (Ta) and niobium (Nb) alloys, are widely used in various fields, but their use in the production of orthopedic implants is limited. Numerous studies and developments are conducted worldwide on various types of metallic biomaterials. According to the literature, the elemental components of Ti, Nb, and Ta metals are generally non-toxic and have a high level of safety and biocompatibility [27]. The titanium alloy BT-6 (Ti-6Al-4V) was chosen as a control group composite alloy because BT-6 (Ti-6Al-4V) is one of the common combinations of biocompatible alloys used in the production of biomedical implants worldwide. There is no comprehensive research on the combination of TNT (Ti21Nb6Ta) according to literature data. This combination may improve the outcomes of surgical treatment due to enhanced product properties.

Thus, the results of the MTT test showed that aqueous extracts from metal alloys TNT and BT-6 do not exhibit toxic effects on fibroblasts and human periosteal cells, as the cell viability exceeded 50%.

Thus, it can be assumed that aqueous extracts from TNT and BT-6 metal alloys do not exert a toxic effect on fibroblasts and human periosteal cells, as cell viability exceeded 50%. In similar experiments, Jian Xu et al. (2013) in their study of the Ti-25Nb alloy showed, using the MTT test, that extracts from the Ti-25Nb alloy did not exhibit toxic effects on rabbit bone marrow mesenchymal stem cells, emphasizing the favorable biocompatibility of the alloy, with cell growth ranging from 94% to 113% [28]. Shimaa El-Hadad et al. (2018), using the MTT test, determined the viability of Saos-2 bone cells after incubation with Ti-6Al-4V alloy samples, which demonstrated a relatively strong proliferative effect on cells, indicating high cytocompatibility. Notably, samples obtained from Ti-6Al-7Nb demonstrated high cytotoxicity, with cell viability below 50%, suggesting that this combination of metal alloys may not be suitable for implantation due to its toxicity [29].

E. Dayaghi et al. (2019) tested a magnesium-zinc framework with tetracycline at different concentrations of tetracycline (MnZn-xTC – 1%, 5%, 10%) in interaction with human bone osteosarcoma cells. According to the study results, frameworks consisting of MnZn and MnZn-xTC with the inclusion of 1% and 5% tetracycline exhibited biocompatibility. However, the MZ-10TC framework with a higher concentration of tetracycline demonstrated toxicity [30].

The analysis of toxic characteristics showed that water extracts of TNT and BT-6 metals lack in vitro hemolytic activity on isolated rat erythrocytes, with hemolysis being absent and staying within acceptable limits of no more than 2%.

In our research, we found that the hemolytic coefficients for TNT and BT-6 samples were 0.379% and 0.372%, respectively, with permissible values of not more than 2%. The obtained p-value was 0.12, and at a 95% probability threshold ($p<0.05$), there were no statistically significant differences in the groups of metal extracts compared to the negative control group (hemolysis 0.9% NaCl). The examination of toxic properties showed that aqueous extracts from TNT and BT-6 metals do not possess in vitro hemolytic activity when acting on isolated erythrocytes of experimental animals (hemolysis was absent). For example, Jialin Niu et al. (2013) studied erythrocyte hemolysis in interaction with an Mg–Nd–Zn–Zr alloy with brushite coating, and the samples with brushite coating showed higher hemocompatibility – 0.68%, compared to the uncoated sample – 48% [31].

The outcomes of the LAL test indicated that the examined water extracts from metal alloys TNT and BT-6 are non-pyrogenic, given the bacterial endotoxin level of 0.05 EU/ml, aligning with the absence of pyrogenic properties in TNT and BT-6 alloys.

Conclusions

The cytotoxicity results indicate that aqueous extracts from TNT and BT-6 metal alloys do not exert a toxic effect on fibroblasts and human periosteal cells under in vitro conditions. The investigation of the toxic properties of TNT and BT-6 metal alloys reveals that aqueous extracts from these materials do not possess hemolytic and pyrogenic activity in vitro.

Our conducted research demonstrates that the new TNT alloy (Ti21Nb6Ta) is biologically safe (non-toxic, hemocompatible, and non-pyrogenic) in vitro. The further strategy for studying the biocompatibility of this alloy is focused on conducting in vivo tests for more thorough examination, aiming for subsequent biomedical applications.

Conflict of interest. The authors declare no conflicts of interest. Sponsors did not participate in the design, collection, analysis, interpretation of data, writing of the manuscript, or the decision to publish the results.

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Authors' contributions. Conceptualization – N.B., O.B., S.P.; methodology – K.O., A.B., D.R.; verification - B.A., V.O.; formal analysis – D.S.; writing (original draft) – A.K.; writing (review and editing) – A.K., B.M.

References

1. Лазарев, А.Ф., Солод Э.И., Ахтямов И.Ф. Рациональный остеосинтез / Монография. - Казань. Скрипта. – 2011. – С.287. [Google Scholar]

Lazarev, A.F., Solod E.I., Akhtiamov I.F. Ratsional'nyi osteosintez (Rational osteosynthesis) [in Russian]. Monografiia. - Kazan'. Skripta. 2011; 287. [Google Scholar]

2. Минасов Б.Ш., Шустер Л.Ш., Якупов Р.Р., Асланян И.Р. и др. Сравнительные характеристики различных пар трения, используемых при артропластике тазобедренного сустава // Гений ортопедии. – 2014. – №3. – С. 48-55. [Google Scholar]

Minasov B.Sh., Shuster L.Sh., Iakupov R.R., Aslanian I.R. i dr. Sravnitel'nye kharakteristiki razlichnykh par treniia, ispol'zuemykh pri artroplastike tazobedrennogo sustava (Comparative characteristics of various friction pairs used in hip arthroplasty) [in Russian]. Genii ortopedii. 2014; 3: 48-55. [Google Scholar]

3. Набоков А.Ю. Современный остеосинтез / Монография. – Казань. Медицинское информационное агентство. – 2007. – С. 400. [Google Scholar]

Nabokov A.Iu. Sovremennyi osteosintez (Modern osteosynthesis) [in Russian]. Monografiia. – Kazan'. Meditsinskoe informatsionnoe agentstvo. 2007; 400. [Google Scholar]

4. Скороглядов А.В., Атаев Э.А. Комплексное лечение посстравматических нарушений костной регенерации длинных костей конечностей // Лечебное дело. – 2013. – №1. – С. 55-61. [Google Scholar]

Skorogliadov A.V., Ataev E.A. Kompleksnoe lechenie posstravmaticheskikh narushenii kostnoi regeneratsii dlinnykh kostei konechnostei (Complex treatment of post-traumatic disorders of bone regeneration of long bones of the extremities) [in Russian]. Lechebnoe delo. 2013; 1: 55-61. [Google Scholar]

5. Маланин Д.А., Калита В.И., Ланцов Ю.А., Деревянко И.В. и др. Физико-механические свойства раздела между костной тканью и имплантатами с различными биоактивными покрытиями // Бюллетень Волгоградского научного центра РАМН и администрации Волгоградской области. – 2008. – №4(20). – С. 47-50. [Google Scholar]

Malanin D.A., Kalita V.I., Lantsov Iu.A., Derevianko I.V. i dr. Fiziko-mekhanicheskie svoistva razdela mezhdu kostnoi tkan'iu i implantatami s razlichnymi bioaktivnymi pokrytiiami (Physico-mechanical properties of the interface between bone tissue and implants with various bioactive coatings) [in Russian]. Biulleten' Volgogradskogo nauchnogo tsentra RAMN i administratsii Volgogradskoi oblasti. 2008; 4(20): 47-50. [Google Scholar]

6. Попков А.В. Управление репаративной регенерацией и гистогенезом тканей – основной путь профилактики неудовлетворительных исходов лечения пациентов ортопедо–травматологического профиля // Ошибки и осложнения в травматологии и ортопедии. - Омск. – 2011. – С. 36–37.

Popkov A.V. Upravlenie reparativnoi regeneratsiei i gistogenezom tkanei – osnovnoi put' profilaktiki neudovletvoritel'nykh iskhodov lecheniia patsientov ortopedo–travmatologicheskogo profilia (Controlling reparative regeneration and tissue histogenesis is the main way to prevent unsatisfactory treatment outcomes for orthopedic and traumatological patients) [in Russian]. Oshibki i oslozhneniia v travmatologii i ortopedii. - Omsk. 2011; 36–37.

7. Алиев Э.И. Клинико-экспериментальное обоснование применения остеофиксаторов с биоинертным покрытием нитридами титана и гафния / Диссертация на соискание ученой степени кандидата медицинских наук. – 2018. – С. 26. [Google Scholar]

Aliev E.I. Kliniko-eksperimental'noe obosnovanie primeneniia osteofiksatorov s bioinertnym pokrytiem nitridami titana i gafniia (Clinical and experimental rationale for the use of osteofixators with a bioinert coating of titanium and hafnium nitrides) [in Russian]. Dissertatsiia na soiskanie uchenoi stepeni kandidata meditsinskikh nauk. 2018; 26.[Google Scholar]

8. Загородний Н.В., Бухтин К.М., Кудинов О.А., Чрагян Г.А. и др. Реакция на кобальт как причина ревизионного эндопротезирования коленного сустава // Вестник травматологии и ортопедии им. Н.Н. Приорова. – 2013. – №2. – С.65–68. [Google Scholar]

Zagorodnii N.V., Bukhtin K.M., Kudinov O.A., Chragian G.A. i dr. Reaktsiia na kobal't kak prichina revizionnogo endoprotezirovaniia kolennogo sustava (Reaction to cobalt as a reason for revision knee arthroplasty) [in Russian]. Vestnik travmatologii i ortopedii im. N.N. Priorova. 2013; 2: 65–68. [Google Scholar]

9. Summer B., Thomas P. Metal Implant Allergy as a Differential Diagnosis of Periprosthetic Joint Infection. Ceranews. 2014; 2: 12-33. [Google Scholar]

10. Thyssen J.P., Menne T. Metal allergy – a review on exposures, penetration, genetics, prevalence, and clinical implications. Chemical research in toxicology. 2010; 23(2): 309-318. [Crossref]

11. Thomas P., Summer B., Krenn V., Thomsen M. Allergiediagnostik bei Metall Verdacht auf Metallimplantat unverträglichkei. Orthopäde. 2013; 42(8): 602-606. [Crossref]

12. Соколов В.А. Множественные и сочетанные травмы / Практическое руководство для врачей-травматологов. – Москва. ГЭОТАР-Медиа. – 2008. – С. 514. [Google Scholar]

Sokolov V.A. Mnozhestvennye i sochetannye travmy (Multiple and combined injuries) [in Russian]. Prakticheskoe rukovodstvo dlia vrachei-travmatologov. – Moskva. GEOTAR-Media. 2008; 514. [Google Scholar]

13. Ахтямов И.Ф., Шакирова Ф.А., Клюшкина Ю.А., Бакланова Д.А. и др. Анализ регенеративного процесса в области перелома большеберцовой кости (экспериментальное исследование) // Травматология и ортопедия России. – 2016. – Т. 22. - №1. – С.100-107. [Google Scholar]

Akhtiamov I.F., Shakirova F.A., Kliushkina Iu.A., Baklanova D.A. i dr. Analiz regenerativnogo protsessa v oblasti pereloma bol'shebertsovoi kosti (eksperimental'noe issledovanie) (Analysis of the regenerative process in the area of tibial fracture (experimental study)) [in Russian]. Travmatologiia i ortopediia Rossii. 2016; 22(10): 100-107. [Google Scholar]

14. Chen P.S., Tsai P.H., Li T.H., Jang J.S. et al. Development and Fabrication of Biocompatible Ti-Based Bulk Metallic Glass Matrix Composites for Additive Manufacturing. Materials (Basel). 2023; 16(17): 5935. [Crossref]

15. López-Valverde N., Aragoneses J., Rodríguez C., Aragoneses J.M. Effect on osseointegration of dental implants treated with carboxyethylphosphonic acid and functionalized with BMP-2: preliminary study on a minipig model. Front Bioeng Biotechnol. 2023; 11: 1244667. [Crossref]

16. Singh A., Shetty S., Kotian R., Rao P. et al. The Evaluation of Biocompatibility, Elemental Composition and Surface Analysis of Retrieved Infra-zygomatic Screws. J Bio Tribo Corros. 2022; 8: 85. [Crossref]

17. Jian S.Y., Lin C.F., Tsai T.L., Wang P.H. et al. In Vivo Degradation Behavior of Magnesium Alloy for Bone Implants with Improving Biological Activity, Mechanical Properties, and Corrosion Resistance. Int J Mol Sci. 2023; 24(2): 1602. [Crossref]

18. Feng J., Tang Y., Liu J., Zhang P. et al. Bio-high entropy alloys: Progress, challenges, and opportunities. Front Bioeng Biotechnol. 2022; 10: 977282. [Crossref]

19. Lons A., Putman S., Pasquier G., Migaud H. et al. Metallic ion release after knee prosthesis implantation: a prospective study. Int Orthop. 2017; 41(12): 2503-2508. [Crossref]

20. Бунятян Н.Д., Васильев А.Н., Верстакова О.Л., Журавлева М.В. и др. Руководство по проведению доклинических исследований лекарственных средств / Монография. – Москва: Гриф и К. – 2012. – С. 944. [Google Scholar]

Buniatian N.D., Vasil'ev A.N., Verstakova O.L., Zhuravleva M.V. i dr. Rukovodstvo po provedeniiu doklinicheskikh issledovanii lekarstvennykh sredstv (Guidelines for conducting preclinical studies of medicinal products) [in Russian]. Monografiia. Moskva: Grif i K. 2012; 944. [Google Scholar]

21. World Medical Association. World Medical Association Declaration of Helsinki. JAMA. 2013; 310(20): 2191. [Crossref]

22. Krakowian D., Gądarowska D., Daniel-Wójcik A., Mrzyk I. A proposal for a new in vitro method for direct classification of eye irritants by cytotoxicity test - Preliminary study. Toxicol Lett. 2021; 338: 58-66. [Crossref]

23. Межгосударственный стандарт. Изделия медицинские. Оценка биологического действия медицинских изделий. Часть 12. Приготовление проб и контрольные образцы. Веб-сайт [дата обращения 16 мая 2023]. Режим доступа: https://meganorm.ru/Data2/1/4293782/4293782541.pdf

Mezhgosudarstvennyi standart. Izdeliia meditsinskie. Otsenka biologicheskogo deistviia meditsinskikh izdelii. Chast' 12. Prigotovlenie prob i kontrol'nye obraztsy. Veb-sait (Interstate standard. Medical products. Assessment of the biological effects of medical devices. Part 12. Sample preparation and control samples) [in Russian]. [data obrashcheniia 16 maia 2023]. Rezhim dostupa: https://meganorm.ru/Data2/1/4293782/4293782541.pdf

24. Seyfert U.T., Biehl V., Schenk J. In vitro hemocompatibility testing of biomaterials according to the ISO 10993-4. Biomolecular engineering, 2002; 19(2-6): 91-96. [Crossref]

25 ISO 10993-4:2017 specifies general requirements for evaluating the interactions of medical devices with blood. Website. [Cited 30 March 2023]. Available from URL: https://www.iso.org/standard/63448.html

26. Копицына М.Н., Морозов А.С., Бессонов И.В., Писарев В.М. Методы определения бактериального эндотоксина в медицине критических состояний (обзор) // General Reanimatology. – 2017. – Т. 13. – №5. – С.109-120. [Crossref]

Kopicyna M.N., Morozov A.S., Bessonov I.V., Pisarev V.M. Metody opredelenija bakterial'nogo jendotoksina v medicine kriticheskih sostojanij (obzor) (Methods for determining bacterial endotoxin in critical care medicine (review)) [in Russian]. General Reanimatology, 2017; 13(5): 109-120. [Crossref]

27. Alvarado-Hernández F., Mihalcea E., Jimenez O., Macías R. et al. Design of Ti64/Ta Hybrid Materials by Powder Metallurgy Mimicking Bone Structure. Materials. 2023; 16(12): 4372. [Crossref]

28. Xu J., Weng X. J., Wang X., Huang J. Z. et al. Potential use of porous titanium-niobium alloy in orthopedic implants: preparation and experimental study of its biocompatibility in vitro. PloS one. 2013; 8(11): e79289. [Crossref]

29. El-Hadad S., Safwat E.M., Sharaf N.F. In-vitro and in-vivo, cytotoxicity evaluation of cast functionally graded biomaterials for dental implantology. Mater Sci Eng C Mater Biol Appl. 2018; 93: 987-995. [Crossref]

30. Dayaghi E., Bakhsheshi-Rad H.R., Hamzah E., Aziz M.et al. Magnesium-zinc scaffold loaded with tetracycline for tissue engineering application: In vitro cell biology and antibacterial activity assessment. Mater Sci Eng C Mater Biol Appl. 2019; 102: 53-65. [Crossref]

31. Niu J., Yuan G., Liao Y., Mao L. et al. Enhanced biocorrosion resistance and biocompatibility of degradable Mg-Nd-Zn-Zr alloy by brushite coating. Mater Sci Eng C Mater Biol Appl. 2013; 33(8): 4833-4841. [Crossref]

TNT (Ti21Nb6Ta) және ВТ-6 (Ti-6Al-4V) металл қорытпаларының биологиялық қауіпсіздігін бағалау

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Түйіндеме

Травматология және ортопедия салаға батпалы фиксаторлар және жаңа биометаллдық қорытпалар сияқты заманауи технологияларды енгізу, емдеу тәсілдерін сапалы өзгертуге әсер етті, бірақ имплланттарды өндіру, және жаңа материалдарды пайдалану мәселесі өзекті болып қала береді.

Зерттеудің мақсаты: титан, тантал және ниобий қорытпасының (Ti21Nb6Ta) биоүйлесімділігін in vitro зерттеу.

Әдістері. Зерттеуде TNT (Ti21Nb6Ta) және ВТ-6 (Ti-6Al-4V) сулы сығындыларының МТТ-сынағы арқылы in vitro адамның фибробласттары мен периостальды жасушаларына уытты әсері зерттелді; TNT және ВТ-6 қорытпаларының гемолитикалық белсенділігін – тәжірибелік жануарлардың Wistar егеуқұйрықтары қанындағы әсерін спектрофотометрия әдісімен (in vitro) зерттелді; Пирогендік белсенділік кинетикалық хромогендік әдіспен зерттелді. Зерттеу объектісі TNT (Ti21Nb6Ta) және ВТ-6 (Ti-6Al-4V) (бақылау тобы) металл қорытпалары болып табылады.

Нәтижелері. In vitro зерттеулер TNT және ВТ-6 металл қорытпаларынан алынған сулы сығындылардың өсірілген адамның фибробласттарына және in vitro периостальды жасушаларына цитотоксикалық әсер етпейтінін көрсетті. TNT және ВТ-6 қорытпаларының сулы сығындыларының гемолитикалық және пирогендік белсенділігі жоқ екені анықталды.

Қорытынды. Жүргізілген зерттеулер TNT қорытпасының қауіпсіздігі мен биоүйлесімділігінің жоғары деңгейін көрсетеді, бұл in vitro сынақтарының жиынтығымен дәлелденді. Жалпы қабылданған гигиеналық классификацияға сәйкес TNT (Ti21Nb6Ta) және ВТ-6 (Ti-6Al-4V) металл қорытпаларын 4-ші қауіптілік классы – төмен қауіпті заттарға жатқызуға болады (ГОСТ 12.1.007-76).

Түйін сөздер: металл қорытпасы, Ti-Nb-Ta, уыттылық, биоүйлесімділік, гемолиз, пирогенділік.

Оценка биологической безопасности сплавов металлов TNT (Ti21Nb6Ta) и ВТ-6 (Ti-6Al-4V)

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Резюме

Внедрение современных технологий в травматологии и ортопедии, таких как погружные фиксаторы и новые биометаллические сплавы, привело к качественному изменению подходов к лечению, но остается актуальным применение новых материалов при изготовлении имплантатов.

Цель исследования: изучить биосовместимость сплава из титана, тантала и ниобия (Ti21Nb6Ta) в условиях in vitro.

Методы. В исследовании изучались токсические воздействия водных вытяжек из TNT (Ti21Nb6Ta) и ВТ-6 (Ti-6Al-4V) на фибробласты и клетки надкостницы человека в условиях (in vitro) при помощи МТТ-теста; гемолитическая активность сплавов TNT и ВТ-6 на крови экспериментальных животных - крыс породы Wistar (in vitro) методом спектрофотометрии; так же исследовалась пирогенная активность кинетическим хромогенным методом. Объектом исследования являются сплавы металлов TNT (Ti21Nb6Ta) и ВТ-6 (Ti-6Al-4V) (группа контроля).

Результаты. Исследования in vitro показали, что водные вытяжки из сплавов металлов TNT и ВТ-6 не оказывают цитотоксического действия на культивируемые фибробласты и клетки надкостницы человека в условиях in vitro. Выявлено, что водные вытяжки сплавов TNT и ВТ-6 не обладают гемолитической и пирогенной активностью.

Выводы. Проведенные исследования свидетельствуют о высоком уровне безопасности и биосовместимости сплава TNT, что доказано комплексом проведенных in vitro тестов. Таким образом, по общепринятой гигиенической классификации сплавы металлов TNT (Ti21Nb6Ta) и ВТ-6 (Ti-6Al-4V) можно отнести к 4 классу опасности – малоопасным веществам (ГОСТ 12.1.007-76).

Ключевые слова: сплав металла, Ti-Nb-Ta, токсичность, биосовместимость, гемолиз, пирогенность.