



***In vivo* biological testing of titanium alloys with added rare earth elements to assess their possible use in medical products**

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Abstract

Introduction Medical implants for treating injuries and orthopedic diseases are often made of titanium and its alloys. Their physicochemical properties, including corrosion inhibition, can be improved by adding rare earth elements.

The **aim** of this study was to evaluate the safety of new materials based on the titanium alloy Ti6Al7Nb doped with yttrium, lanthanum, and cerium using an experimental *in vivo* subcutaneous implantation model.

Materials and Methods Male Wistar rats were subcutaneously implanted with titanium and titanium alloy samples: VT1-00 (control, $n = 10$); Ti6Al7Nb0.3Y (group 1, $n = 12$); Ti6Al7Nb0.3La (group 2, $n = 12$); Ti6Al7Nb0.3Ce (group 3, $n = 12$). The experiment lasted 28 days. The animals' general condition and behavioral responses were assessed, and the implantation area was visually marked. Body weight, body temperature, and local temperature at the implantation site were recorded. Hematological and biochemical blood tests were performed, and internal organs and peri-implant tissue condition were anatomically assessed.

Results In all groups, general condition, behavioral responses, body weight, body temperature, and peri-implant tissue temperature were normal, and skin wound healing occurred by primary intention. A positive effect of the rare earth elements studied was observed on reparative processes during skin wound healing. In the control group and group 1, organs retained normal size, color, and anatomical structure. In group 1, red blood cell counts were slightly elevated, along with increased concentrations of low- and medium-molecular-weight substances. In groups 2 and 3, changes in the anatomical characteristics of the liver, kidneys, and spleen were determined. Serum AST and LDH levels increased, C-reactive protein levels decreased, the proportion of neutrophils increased, and the lymphocyte count decreased. Glucose levels decreased in group 2, while glucose and urea levels increased in group 3.

Discussion Subcutaneous implantation of yttrium (Y), lanthanum (La), and cerium (Ce) at 0.3 % wt. each in titanium alloys of Ti6Al7Nb composition for one month had no negative impact on the general condition, thermoregulation, cardiovascular system, or reproductive organs of male rats. The titanium alloy doped with yttrium (Y) had a compensatory toxic effect on the body. Titanium alloys doped with lanthanum (La) and cerium (Ce) exhibited hepatotoxic and nephrotoxic effects and impaired spleen function. The results obtained are consistent with existing literature data.

Conclusion Under the conditions created, yttrium-doped materials and control samples can be considered safe. Materials doped with lanthanum and cerium raise concerns when implanted *in vivo*, requiring a longer-term study using histological methods.

Keywords: traumatology and orthopedics, implant, titanium alloy, corrosion, yttrium, lanthanum, cerium, *in vivo* experiment, medical product safety

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INTRODUCTION

It is known that some conventional metal alloys used in the manufacture of implantable medical product, including those in additive manufacturing, have a number of shortcomings that can negatively impact treatment outcomes, particularly in patients with musculoskeletal disorders. This is typically due to the physicochemical properties of the materials themselves, whose elastic modulus (Young's modulus) often significantly exceeds that of bone tissue [1–3]. Under such conditions, in prolonged implantation, bone loss occurs at the interface between the implant surface and the bone, leading to fixation instability, the elimination of which requires repeated surgery. A number of elements included in metal alloys have a toxic effect on surrounding tissues and the body as a whole. To prevent such effects, materials must have increased corrosion resistance [4–6].

Currently, research is being conducted to improve the mechanical properties of titanium and its alloys, in particular. They are most frequently used in the manufacture of medical products, including those intended for traumatology and orthopedics [3, 7, 8]. One method for enhancing mechanical properties, wear resistance, and corrosion resistance is alloying such materials with rare earth elements (REEs). Thus, the addition of a small amount of yttrium or cerium (0.3 wt. %) reduces the elastic modulus and improves plasticity. The corrosion rate is reduced by the addition of lanthanum, cerium, yttrium, or neodymium. While low concentrations of rare earth metals often improve the characteristics of alloys, higher weight fractions of these elements can negatively influence porosity, tensile strength, tensile toughness, and other properties [9–11].

Contemporary materials used for the manufacture of medical products, in addition to improved mechanical properties, must possess biocompatible, bioactive, and antibacterial properties [12–16]. Some metals, including rare earth metals, can exhibit antimicrobial activity, which expands the possibilities and justifies their use in the development of new medical alloys [17–19].

Despite the broad potential for using rare earth elements to improve the physicochemical and mechanical properties of alloys intended for the subsequent manufacture of medical devices, their safety has not been sufficiently studied and requires additional comprehensive investigation.

The **aim** of this study was to evaluate the safety of new materials based on the titanium alloy Ti6Al7Nb alloyed with yttrium, lanthanum, and cerium using an experimental *in vivo* subcutaneous implantation model.

MATERIALS AND METHODS

Eight-month-old male Wistar rats with an average body weight of 473.3 ± 74.03 g were used. All animals underwent subcutaneous implantation of test samples of the studied materials in the spine area under operating conditions. The samples were cylindrical, 10.0 mm in diameter, and 1.0 mm thick. The rare earth element content in the samples of the study groups was 0.3 wt. %.

Four groups of experiments (one control and three study ones) were performed *in vivo*:

- control group — samples made of titanium VT1-00 ($n = 10$);
- study group 1 — samples made of Ti6Al7Nb0.3Y ($n = 12$);
- study group 2 — samples made of Ti6Al7Nb0.3La ($n = 12$);
- study group 3 — samples made of Ti6Al7Nb0.3Ce ($n = 12$).

The procedure was performed in an operating room under general anesthesia adapted to the animals' weight (Rometa 2 % 1–2 mg/kg (Bioveta, Czech Republic); Zoletil 100 10–15 mg/kg (Virbac Sante Animale, France)). After surgery, the animals were placed in cages for two animals. Postoperative analgesia was achieved with Analgin (Biosintez, OJSC, Russia) administered subcutaneously at a dose

of 30–50 mg/kg twice daily for three days. The recommended temperature regime (24–26 °C) was maintained in the rat vivarium throughout the experiment. The animals were kept on a standard balanced diet with free access to water.

To assess the safety of the test materials using *in vivo* testing methods, the animals' body weight (g), overall body temperature (°C), and local body temperature at the implantation site (°C) were recorded immediately before the experiment and at seven, 14, 21, and 28 days postoperatively. The values obtained before surgery were used as the reference values.

At the same time-points, the animals' appearance, behavioral responses, intensity and pattern of motor activity, condition of their hair and skin, and the color of their mucous membranes were examined. A visual assessment of the soft tissues in the implantation area was performed.

The studies were conducted in the morning before the first feeding. After 28 days of the experiment, six animals from each group, randomly selected, were euthanized by decapitation to collect blood for hematological and biochemical analysis. Next, anatomical specimens were collected, and autopsies were performed to remove internal organs. When conducting preclinical studies, especially those aimed at identifying toxic effects, measuring the weight of internal organs and calculating their mass coefficients relative to body weight are mandatory. Analysis of these parameters allows for the detection of the target organ by the toxic agent. Therefore, after removal of the internal organs, they were weighed and their mass coefficients were calculated.

The condition of the tissues around the implant was visually assessed, as well as the location, color, and shape of the internal organs in the chest and abdominal cavities. The remaining animals (four in the control group and six in each experimental group) were retained for long-term study.

For histological study, skin fragments were collected from the implantation area, fixed in 10 % neutral formalin, and embedded in paraffin. Histological sections were prepared using a sled microtome (Reichard, Germany) and then stained with hematoxylin and eosin. Digital images of the histological specimens were obtained by scanning them at a resolution of 40x using a PANNORAMIC Midi II BF scanning microscope for laboratory research (3DHISTECH Ltd., Hungary). The thickness of the epidermis and dermis as well as the area and length of the scar were measured using PANNORAMIC Viewer, version 2.4 (3DHISTECH Ltd., Hungary). The numerical density of derivatives and vessels in the scar area was determined in the field of view of the histological specimen image at a magnification of $\times 400$ and then proportionally recalculated per 1 mm².

In hematological tests, the cell composition of the peripheral blood was determined using standard cell counting techniques in a Goryaev chamber. The counts of leukocytes, erythrocytes, and platelets, as well as the relative numbers of neutrophils, eosinophils, monocytes, and lymphocytes, were determined in the rats' peripheral blood. The white blood cell count was determined in Romanovsky-Giemsa-stained blood smears under immersion at magnification $\times 100$.

The concentration of total protein, urea, creatinine, glucose, C-reactive protein (CRP), as well as the activity of aminotransferases (ALT, AST) and lactate dehydrogenase (LDH) were determined in the blood serum of experimental animals. Biochemical studies were performed on an automatic biochemical analyzer Hitachi/BM 902 (F. Hoffmann-La Roche Ltd., Italy), using reagent kits from Vital Diagnostics, Vector-Best, BioSystems (Russia). The level of endogenous intoxication was assessed by the content of low- and medium-molecular-weight substances (LMMW) in the blood serum, among which the percentage of the catabolic pool (Cat. %) was calculated, equal to the sum of decay products recorded in the wavelength range of 238–258 nm. The content of LMMW was determined using the method of Malakhova [20].

Animal body weight was recorded using a TV A electronic scales. A POCKET SCALE MH-100 portable electronic scales (100 g/0.01 g) was used to weigh internal organs. Body temperature was measured using a DT-622 electronic thermometer, and local body temperature was measured using a Uni-T Uti320e infrared thermal imager (Fig. 1).

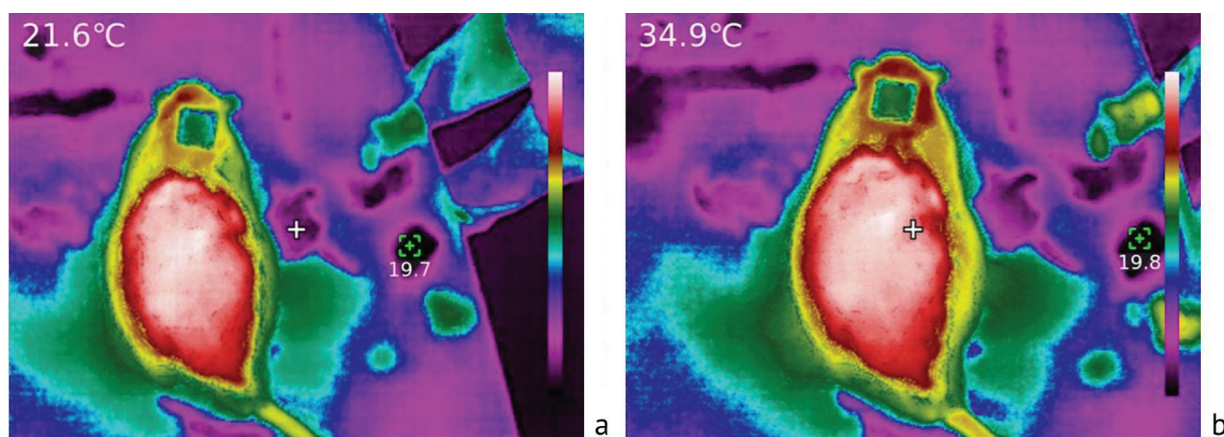


Fig. 1 Thermogram of the spinal area of the experimental animal: (a) immediately before the thermal imaging examination; (b) during the thermal imaging examination (the cross marks the area of implantation of the test sample from which the data was collected; in the upper left corner is the temperature value of the integumentary tissues of the implantation area of the test sample)

The obtained quantitative data were subjected to statistical analysis. Statistical analysis was performed using the AtteStat version 13.1 add-in for Excel spreadsheets (2016, 16.0.5278.1000). Descriptive statistics were used to determine the median and interquartile range (Me, Q1–Q3). The Wilcoxon test was used to compare the dynamics of changes within a one group (dependent samples). The Kruskal-Wallis test was used to compare indicators between the study groups and the control group (independent samples). Differences were considered statistically significant at $p < 0.05$.

The animals were cared for in accordance with the requirements of GOST R 33044-2014 "Principles of Good Laboratory Practice", GOST 33215-2014 "Guidelines for the Care and Maintenance of Laboratory Animals. Rules for the Equipment of Premises and Organization of Procedures", GOST 33216-2014 "Guidelines for the Care and Maintenance of Laboratory Animals. Rules for the Care and Maintenance of Laboratory Rodents and Rabbits". The experiments were carried out in accordance with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes and Directive 2010/63/EU of the European Parliament and of the Council of the European Union of 22.09.2010 on the protection of animals used for scientific purposes. The study was approved by the Ethics Committee of the IMET RAS (Protocol No. 1 of 15.02.2024).

RESULTS

Throughout the experiment, the general condition of the animals in all groups was satisfactory. No deaths were recorded. Neither deviations in food or water consumption, nor any infectious or neurological complications were observed. Skin and hair condition, as well as mucous membrane coloration, were unchanged. Animal behavior and general condition were consistent with the expected clinical picture. At rest and during movement, the animals assumed a natural physiological position.

In none of the groups at different time-points of the experiment there were no significant changes in body weight in relation to the initial values.

In all groups, at all time-points of the experiment, a significant decrease in overall body temperature was recorded compared to preoperative values. No significant differences were found between the groups.

The analysis of local body temperature dynamics at the implantation site of the test samples revealed that after seven days of the experiment, this parameter significantly decreased in the control group and group 1 compared to baseline values by 1.6°C ($p = 0.0002$; $p = 0.01$, respectively). During this period, local temperature in group 2 was significantly higher than in the other groups by 0.9–1.2 °C ($p < 0.05$). Subsequently, this parameter was significantly lower than baseline values in all groups, but no differences were found within or between groups.

The dynamics of changes in body weight, general body temperature and local tissue temperature in the area of implantation of test samples are presented in Table 1.

Table 1

Changes in body weight, general and local body temperature, Me (Q1–Q3)

Groups	Experiment stages				
	0 days (norm)	7 days	14 days	21 days	28 days
Body weight, g					
Control	412 (390–434)	404 (388–439)	393 (378–439)	394 (382–452)	398 (386–398)
1	440 (417–461)	435.5 (426–479)	440 (411–427)	429 (409–435,5)	424 (410–434)
2	523 (446–574)	528 (348–540)	542 (496–558)	560 (514–584)	556 (526–574)
3	552 (507–591)	565 (485–582)	564 (499–584)	577 (502–588)	560 (510–592)
General body temperature, °C					
Control	37.3 (37.2–37.6)	36.35 (36.3–36.7)	36.65 (36.2–37)	36.5 (36.3–36.5)	36.2 (35.8–36,2)
1	37.1 (36.65–37.55)	36.35 (36.15–36.65)	36.4 (36.2–36.35)	36.3 (36.05–36.35)	36.5 (36.1–36.5)
2	37.7 (37.55–37.95)	36.65 (36.4–36.75)	36.5 (36.4–36.5)	36.65 (36.4–36.9)	36.8 (36.6–37)
3	37.2 (37.1–37.35)	36.6 (36.45–36.8)	36.5 (36.25–36.95)	36.6 (36.4–36.8)	36.7 (36.5–36.8)
Local body temperature, °C					
Control	34.1 (34–34.3)	32.5 (32.1–32.9)	32 (31.2–32)	33 (32–33)	32.5 (31.8–32.5)
1	33.75(33.05–34.2)	32.1 (31.35–33.1)	32.6 (32.15–32.4)	32.1 (31.65–32.1)	32.8 (32.1–32.8)
2	34.95 (34.05–35)	34.2* (33.55–34.85)	32.0 (31–32.8)	32.5 (32–33.8)	33.0 (32.2–33.2)
3	34.0(33.8–34.2)	33.3(32.3–34.1)	32.55 (32.05–33.3)	32.9 (31.95–33.1)	32.7 (31.55–33.45)

Notes: **bold typed** are values that differ statistically from the preoperational ones (0 days) at $p < 0.05$; * are differences of the parameter with the control, study group 1 and study group 3 at $p < 0.05$.

The postoperative wound at the site of implantation of the tested specimen healed by primary intention in all experiment groups. No hematomas, abscesses, or other pathological changes in the skin were observed at any stage of the experiment. During anatomical preparation, no local signs of inflammation were visually observed in the area of contact between the test specimens and the adjacent soft tissue.

In the control group (BT1-00), skin wound healing after implantation occurred by primary intention, under a scab. Seven days later, a brown "crust" was visible on the surface of the skin wound. No swelling of the surrounding tissues or hemorrhages in the adjacent intact skin tissues was observed. After 14 days, complete or almost complete desquamation of the scab occurred. Epithelization of the wound surface was observed. After 21 days, a normotrophic linear scar with signs of remodeling was observed in the area of skin damage. Suture marks were visible. After 28 days, an even greater restructuring of the scar tissue into normotrophic tissue occurred. The scar in the healing area had a linear shape and was hardly visualized. Dissection of the skin flap around the implanted sample provoked the formation of a fairly dense fibrous capsule (Fig. 2a). No signs of significant edema, hemorrhage, ulceration, or necrosis were detected in the fibrous capsule tissue. Minor thickening and compaction of the subcutaneous and superficial muscular fascia were detected.

In Group 1 (Ti6Al7Nb0.3Y), healing occurred under a scab. However, unlike in the control group, complete wound epithelialization had not occurred after 14 days, with the scab partially peeling off. During this period, slight swelling of the tissues surrounding the skin wound was observed, manifested by a mound-shaped protrusion of tissue around the healing skin wound above the general horizontal skin surface. However, by the 21st day, a filiform linear scar had formed, which by the 28th day was virtually invisible (Fig. 2b). The surrounding tissues showed no visual changes. A thin, transparent connective tissue capsule formed around the implant (Fig. 2b). No reactions of the surrounding tissues to the implant were observed. The fascia were not thickened, translucent, and organotypic.

After seven days of the experiment, in Group 2 (Ti6Al7Nb0.3La), the skin wound surface was covered with a tan-colored scab. Labium-like swelling of the skin wound edges was noted, which persisted through the 14th day of observation. No changes in the color of the skin adjacent to the wound were visible. No significant localized edema was detected. By the 14th day, the scab did not sloughed off and covered the entire wound surface. By the 21st day, wound epithelialization occurred, and the scab was no longer detectable. The formation of thin epithelium at the site of skin injury was confirmed by the intense pink color of the linear scar that had formed by this time. The scar was structurally hypotrophic. After 28 days, the scar was hardly visible at external examination. A translucent fibrous tissue was formed around the subcutaneous implant. The fascial tissue surrounding the implant lost its transparency, became denser and significantly thicker, and acquired a whitish-flesh-colored appearance and a slight opacity (Fig. 1c).

In Group 3 (Ti6Al7Nb0.3Ce), similarly to the other groups, wound healing occurred under the scab by primary intention. By the seventh day of the experiment, the wound edges were tightly closed, and the scab, unlike in the other series, was already beginning to peel off. No wound discharge was observed, as in the other groups. By the 14th day,

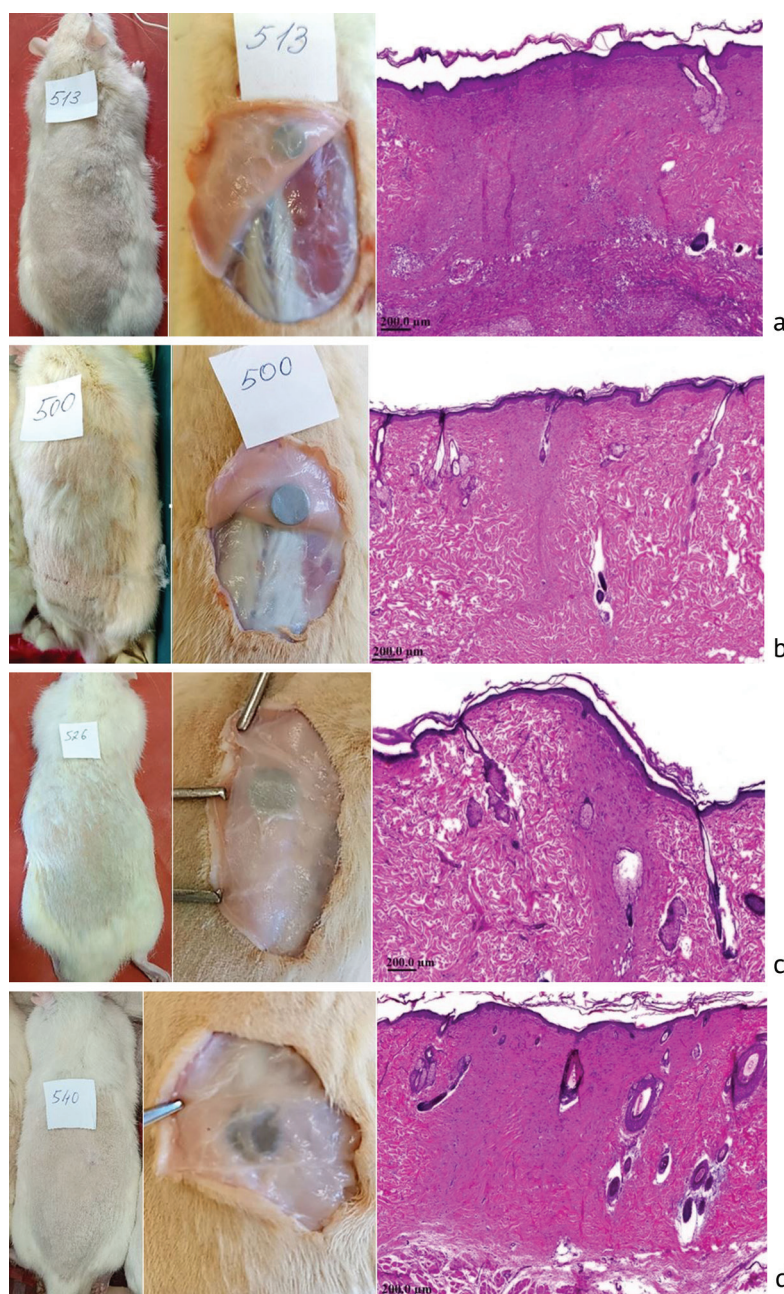


Fig. 2 The condition of the tissues in the implantation area after 28 days of the experiment: (a) control group (VT1-00); (b) group 1 (Ti6AL7Nb0.3Y); (c) group 2 (Ti6AL7Nb0.3La); (d) group 3 (Ti6AL7Nb0.3Ce). The left column: the appearance of the implantation area; the middle column: the condition of the tissues in the peri-implantation area; the right column: the histostructural organization of the skin scar, stained with hematoxylin and eosin, magnification — 50×

the wound was largely epithelialized, although some of the scab remained. The wound tissue did not protrude above the skin surface. No significant swelling, hemorrhage, or tissue necrosis was observed. After 21 days, a hypotrophic scar with labium-like protrusions along the wound edges was formed. After 28 days, the scar became less noticeable, while its hypotrophic nature persisted. The implant was surrounded by a dense, opaque, and fairly thick fibrous capsule at its periphery, becoming thinner, more transparent, and more heterogeneous in the central areas above the implant surface. The surrounding tissues were fairly dense, opaque, and flesh-white in color, with fatty inclusions (Fig. 1d).

Histologically, by the 28th day of the experiment, the formation of a skin regenerate (scar) was observed in the area of the skin wound in both the treatment and control groups that was covered externally by a full-thickness epidermis (Fig. 2a–d, right column). The differences consisted in the fact that in the treatment groups, the cells of the spinous layer were usually arranged in a single row, while in the control group they were in two or even three rows. This was reflected in the thickness of the epidermis, the thickness of which in group 1 was significantly lower than in the control group by 37.4 %, and in groups 2 and 3 — by 49.3 % and 47.3 %, respectively (Table 2). The dermis in the control group (Fig. 2a, right) and group 3 (Fig. 2d, right) was represented predominantly by densely packed fibrous connective tissue (mainly fibrous), the fibers of which lay in different directions. Increased cellularity was observed in group 3. Granulation areas were noted in the control group. Scar length in these groups was greatest compared to groups 1 and 2, exceeding by an average of three times (Table 2). The scar area in the control group was three times greater than in group 3 and nine times greater than in groups 1 and 2. The minimal content of scar tissue was characteristic of the study groups 1 and 2. Dermal thickness in the animals of the control group did not differ significantly from that in group 1, while in groups 2 and 3 it was significantly lower than in the control group.

Table 2

Histological parameters of skin regenerate in the damaged area on the 28th day after implantation of the material, Me (Q1–Q3)

Parameters	Experimental groups			
	Control	Group 1	Group 2	Group 3
Epidermal thickness h, μm	98.8 (96.7–101.4)	62.1 (61.3–70.4)	50.1 (47.1–52.6)	52.1 (47–54.7)
Dermis thickness h, μm	1870.6 (1856.6–1868.3)	1876.8 (1758.3–1876.8)	1272.95 (1171.5–1345.1)	1553.45 (1505.1–1555.7)
Scar length L, μm	1789 (1776–1801)	558.1 (501.3–600.9)	549 (538.6–593.3)	1431.5 (1228.1–1626.5)
Scar area S, mm^2	5.4 (4.9–5.6)	0.553 (0.531–0.584)	0.631 (0.579–0.649)	1.8 (1.6–1.9)
Numerical density of Nader derivative, units/ mm^2	4.75 (4.1–6.2)	7.2 (4.3–9.1)	7.4 (5.8–8.3)	15.6 (16.4–17.03)
Numerical density of vessels NASos, units/ mm^2	59.02 (29.51–59.02)	59.03 (44.26–73.7)	59.02 (29.52–59.01)	59 (29.63–59.6)

Note: **bold typed** are values that differ statistically from the control group (at $p < 0.05$).

The skin regenerates of the control group contained a few hair follicles and sebaceous glands. Their numbers were significantly higher in the regenerates of animals from the experimental groups: by 51.6 % and 55.8 % in groups 1 and 2, respectively, and by 2.28 times in group 3. The density of vessels in the skin regenerates of all groups was similar.

In all groups, including the control group, low leukocyte counts were recorded on the 28th day of the experiment compared to the physiological norm for animals. In group 1, the erythrocyte count significantly increased compared to the results obtained in the control group. Platelet counts in both the study and control groups were within normal limits (Table 3). The blood profile was lymphocytic. The leukogram values in the control group varied within the normal range. In the leukocyte formulas of groups 2 and 3, the number of lymphocytes and neutrophils was significantly different from the values of the control group (Table 3).

Table 3

Hematological parameters of the blood of experimental animals on the 28th day after implantation of the material, Me (Q1–Q3)

Parameter	Reference interval	Control	Group 1	Group 2	Group 3
Erythrocytes, 10 ¹² /l	5.5–11.0	6.6 (6.4–6.8)	7.4 (6.9–7.6)	5.7 (5.5–6.5)	7.0 (6.4–7.5)
Leukocytes, 10 ⁹ /l	8.0–23.0	5.0 (4.7–5.4)	5.2 (4.5–6.2)	5.4 (5.0–5.5)	5.7 (5.0–6.0)
Platelets, 10 ⁹ /l	200–600	430.0 (420.1–490.0)	425.5 (412.5–447.5)	475.5 (425.0–550.0)	475.0 (369.0–675.0)
NEU, %	20–35	29 (28–30)	34 (29–38)	44 (42–45)	44 (34–50)
LYM, %	55–75	65 (64–68)	57 (52–65)	50 (48–52)	49 (44–55)
MON, %	1–5	5 (4–6)	6 (4–6)	3 (3–5)	4 (3–6)
EOS, %	1–5	1 (1–4)	3.5 (2–4.5)	3 (2–3)	3 (3–4)
BAS, %	0–1	0 (0–0)	0.5 (0–0.5)	0 (0–0)	0 (0–0.5)

Note: values in **bold** are significantly different from the values of the control group animals (at $p < 0.05$), NEU – neutrophils, LYM – lymphocytes, MON – monocytes, EOS – eosinophils, BAS – basophils.

The results of the biochemical study of the blood serum (Table 4) showed that 28 days after implantation, the level of endogenous intoxication products (EIPPs) in rats of group 1 was significantly ($p = 0.05$) elevated compared to the animals of the control group. The level of catabolism products in the animals of the study groups did not differ from the control. In groups 2 and 3, the CRP level compared to the control was significantly lower ($p = 0.03$; $p = 0.04$). This indicates that the acute response to the implantation of these materials was lower than that of the control material. A significant change in liver damage markers (AST and LDH) was recorded in rats of group 2 ($p = 0.05$) and group 3 ($p = 0.03$) compared to the control. Moreover, a more significant increase in the activity of these enzymes was noted in the rats of group 3. In animals of this group, a statistically significant ($p = 0.02$) increase in the level of urea in the blood was also noted, as a sign of a nephrotoxic effect. By this time, animals in group 2 showed a significant decrease in blood glucose levels ($p = 0.05$) relative to the animals in the control group.

Table 4

Biochemical parameters of blood serum of experimental animals on the 28th day after implantation of the material, Me (Q1–Q3)

Parameters	Control	Group 1	Group 2	Group 3
Total protein, g/l	67 (64–68)	67 (65–68)	64 (64–68)	67 (62–67)
CRP, µg/L	7.3 (6.2–8.3)	6.7 (5.0–7.9)	2.0 (1.9–3.9)	3.5 (2.7–4.3)
ALT, units/L	67 (58–71)	69 (59–85)	87 (70–91)	85 (70–105)
AST, units/L	121 (117–149)	142 (130–155)	160 (153–195)	158 (155–180)
LDH, units/L	1061 (987–1645)	1547 (1418–2002)	2371 (2230–3045)	2974 (2485–3333)
Urea, mmol/l	4.8 (4.6–5.1)	4.9 (4.8–5.1)	4.9 (4.8–5.6)	6.8 (6.6–7.1)
Creatinine, µmol/l	56 (53–56)	60 (56–61)	49 (48–55)	51 (50–54)
Glucose, mmol/l	21.6 (19.6–23.0)	21.7 (18.9–24.3)	16.7 (16.6–16.9)	22.5 (21.2–23.7)
Low and middle molecular weight substances, units of optical density	7.44 (6.94–7.60)	8.48 (7.95–11.18)	8.22 (7.70–8.27)	7.60 (7.45–8.28)
Cat. %	16.6 (16.4–18.9)	20.6 (15.6–26.2)	15.7 (15.2–16.4)	16.7 (15.7–17.1)

Note: **bold typed** are values that differ statistically from control group (at $p < 0.05$).

The autopsy showed that the internal organs were anatomically arranged in all animals. No significant differences were found between the groups in heart and testicle weight, their color or shape. In group 1, the weight and anatomical characteristics of the liver, spleen, and kidneys were consistent with those of the controls. Macroscopically, liver changes were observed in groups 2 and 3, characterized by smoothed edges and a more saturated brown color. Three of six animals in group 2 euthanized during this period and four of six animals in group 3 had hepatomegaly. Kidney changes were visually detected in these groups: they lacked the typical bean-shaped form

(they were closer to oval), had a more watery structure, and their weight was significantly greater than in the controls ($p = 0.02$). Significant differences in spleen weight ($p = 0.01$) were also observed compared to the same parameter for this organ in the control group. In some cases, visual edema was detected in the center of the organ while maintaining the anatomy of the edges. The weights of the major internal organs are presented in Table 5.

Table 5

Weight of the main internal organs, Me (Q1–Q3)

Groups	Organs (g/% of total body weight)				
	Heart	Liver	Spleen	Kidneys	Testicles
Control	1.75 / 0.45 (1.69–1.79 / 0.42–0.48)	18.86 / 3.76 (17.68–20.83 / 3.44–4.35)	1.03 / 0.26 (0.99–1.09 / 0.25–0.28)	2.45 / 0.66 (2.26–2.56 / 0.61–0.67)	2.59 / 0.66 (2.54–2.71 / 0.64–0.74)
Group 1	1.95 / 0.46 (1.9–1.97 / 0.43–0.47)	19.21 / 4.21 (17.77–25.88 / 3.67–5.5)	1.23 / 0.28 (1.08–1.33 / 0.24–0.32)	2.53 / 0.61 (2.32–2.88 / 0.57–0.65)	2.74 / 0.63 (2.54–2.93 / 0.59–0.68)
Group 2	2.36 / 0.45 (1.94–2.16 / 0.41–0.43)	22.73 / 4.32 (18.17–21.27 / 4.17–4.25)	1.55 / 0.33 (1.36–1.5 / 0.29–0.31)	2.68 / 0.55 (2.45–3.24 / 0.52–0.57)	3.31 / 0.66 (2.89–3.54 / 0.58–0.7)
Group 3	2.49 / 0.45 (2.22–2.56 / 0.43–0.49)	23.27 / 4.04 (17.02–20.81 / 3.98–4.03)	1.68 / 0.32 (1.48–1.86 / 0.29–0.35)	2.99 / 0.55 (2.57–3.47 / 0.51–0.61)	3.19 / 0.6 (3.11–3.5 / 0.57–0.66)

Note: bold typed are values that differ statistically from the control group (at $p < 0.05$).

DISCUSSION

Currently, active research is underway to improve the physicochemical properties of metals, particularly those intended for the manufacture of medical implantable orthopedic products [16, 21, 22]. Ensuring their safety and efficacy requires *in vitro* studies, including mechanical tests to assess corrosion resistance, wear resistance, elasticity, and other characteristics. An important step is testing new alloys *in vivo* using intravital and postmortem methods. They allow for determination of biocompatibility with surrounding tissues, evaluation of the immune response, cytotoxicity, and other possible reactions of a living organism in response to interaction with new materials.

The primary objective of the *in vivo* study was to evaluate the safety of new materials based on titanium alloy doped with rare earth elements such as yttrium, lanthanum, and cerium. The Ti6Al7Nb titanium alloy contained 0.3 wt. % of each of these elements. The test system consisted of mature, clinically healthy male Wistar rats in which the test materials were implanted subcutaneously. The animals were studied for 28 days.

Throughout the experiment, no disturbances in thermoregulation were observed in any of the experimental animals, as evidenced by the absence of significant fluctuations in general and local body temperature in the control and study groups under identical housing conditions. Feed and water intake were also unchanged. Motor and behavioral responses were maintained. No animal deaths were recorded.

In all groups, skin wound healing at the implantation site occurred by primary intention, under a scab. Earlier skin scar formation was observed in group 3 animals with cerium-doped samples. In these animals, scab desquamation was observed by the seventh day of the experiment, whereas in the other groups, the scab covered the entire wound surface by that time. Histological examination revealed a threefold reduction in scar tissue compared to the control group at the skin wound site by the 28th day of the experiment, indicating the effect of cerium on accelerating the organotypic remodeling of scar tissue. Cerium also demonstrated a positive effect on the formation of skin derivatives at the site of injury. Some authors obtained similar results in *in vivo* experiments on male Wistar rats with topical application of cerium oxide nanoparticles in a water-soluble hydrogel to a skin wound. In those studies, complete wound healing with the formation of a sufficiently

strong scar connection with a favorable cosmetic effect was determined as early as the sixth day of the experiment [23]. According to other data, exposure to nanodispersed cerium dioxide activated marginal epithelialization of an experimental burn wound in rats [24]. This effect may be due to the fact that cerium nanoparticles can act as direct antioxidants, limiting the amount of reactive oxygen species necessary for cell death, particularly in nerve cells [25].

Several studies showed that long-term oral administration of cerium nitrate does not have a toxic effect on reproductive function in female laboratory rats. However, behavioral responses, body weight, and uterine weight were unchanged in the breeding stock [26]. In the study presented on male rats in group 3 (samples with cerium) after 28 days, we also observed no changes in behavioral responses, and body weight and testicular mass ratio did not change significantly compared to the control group.

In our study, hematological and biochemical parameters in the control group (VT6) and group 1 (samples with yttrium) were closest to the normal ones. Group 1 showed a statistically significant (but clinically insignificant) increase in red blood cell counts, as well as an increase in the concentration of low- and medium-molecular-weight substances. Blood tests findings were fully consistent with the anatomical findings. Animals in those groups showed no hepatomegaly or changes in the kidneys or spleen. The organs retained normal size, color, and structure. This indicates that the yttrium-doped alloy has a toxic effect that the body is able to compensate for.

Subcutaneous implantation of titanium alloy samples doped with lanthanum (group 2) and cerium (group 3) revealed signs of toxic effects on the liver, kidneys, and spleen. In most cases, the hepatotoxic effect manifested itself as hepatomegaly, changes in liver color and shape, and a sharp increase in AST and LDH in the serum (especially in group 3). In group 2, the decreased glucose levels could also be a consequence of impaired liver metabolism, which, combined with the aforementioned signs, indicated an even more pronounced hepatotoxic effect than in group 3.

Nephrotoxic manifestations in the animals of groups 2 and 3 were characterized by changes in the anatomical shape and structure of kidneys, reflected in the weight of these organs, which increased compared to the control group implanted with VT1 titanium alloy was implanted. Apparently, the negative impact on the kidneys was more pronounced in group 3, as its animals showed statistically significant elevation in blood glucose and urea levels—key markers of impaired renal nitrogen excretion. As noted above, autopsy confirmed this fact, in addition to changes in the shape and structure of these organs, as well as a significant increase in their weight compared to other groups.

The toxic effect on the spleen in animals of groups 2 and 3 was characterized by a significant increase in its weight and organ mass coefficient, combined with swelling.

In groups 2 and 3, compared to the control group, the decrease in C-reactive protein levels can be explained by general suppression of the immune system or the specific effect of lanthanum and cerium ions on the synthesis of acute-phase proteins, which does not negate the presence of toxic damage. Significant changes in the white blood cell count were recorded in those groups: a significant increase in the proportion of neutrophils and a decrease in lymphocytes. This shift is characteristic of a chronic inflammatory process or toxic stress, indicating a systemic response to the implanted material. Signs of toxic chronic inflammation in the kidneys, pancreas, and testicles of white male rats, which arose due to the inhalation of a substance containing, in particular, lanthanum, were seen by other authors [27]. The literature also contains information about the toxic effects of lanthanum on the function of liver, kidneys, and spleen; negative effects on the immune system and bones were noted [28].

It is evident that implantation of yttrium-doped experimental samples (group 1) also caused a minor toxic effect, characterized by increased levels of endogenous intoxication products. However, this effect can be considered compensated for, as it did not manifest itself in visceral organ dysfunction.

Subcutaneous implantation of lanthanum- and yttrium-containing alloys revealed a significant effect on the restructuring of scar tissue toward organotypic restoration. Yttrium in the implanted material promoted normotrophic scar formation, while lanthanum promoted hypotrophic scar formation.

CONCLUSION

The analysis of the study results showed that within one month of implantation in a living organism:

- titanium alloys of Ti6Al7Nb, **doped with yttrium, lanthanum, or cerium** at 0.3 wt. %, have no noticeable negative impact on the cardiovascular system and reproductive organs, do not disrupt thermoregulation processes, and promote reparative processes in skin wounds;
- titanium alloys of Ti6Al7Nb, **doped with yttrium** 0.3 wt. %, contribute to the manifestation of signs of endogenous intoxication without causing organ damage;
- titanium alloys i6Al7Nb, **doped with lanthanum** at 0.3 wt. %, have a pronounced hepatotoxic effect, a nephrotoxic effect, and impair the function of the spleen;
- titanium alloys Ti6Al7Nb, **doped with cerium** at 0.3 wt. %, have a pronounced nephrotoxic effect, a hepatotoxic effect, and impair the function of the spleen.

The results obtained should be considered in developing new materials intended for the manufacture of medical products.

Conflict of interests Not declared

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