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**Cytocompatibility of a free machining titanium alloy containing
lanthanum**

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Abstract

Titanium alloys like Ti6Al4V are widely used in medical engineering. However, the mechanical and chemical properties of titanium alloys lead to poor machinability, resulting in high production costs of medical products. To improve the machinability of Ti6Al4V, 0.9% of the rare earth element lanthanum (La) was added. The microstructure, the mechanical and the corrosion properties were determined. Lanthanum containing alloys exhibited discrete particles of cubic lanthanum. The mechanical properties and corrosion resistance were slightly decreased but are still sufficient for many applications in the field of medical engineering. *In vitro* experiments with mouse macrophages (RAW 264.7) and human bone-derived cells (MG-63, HBDC) were performed and revealed that macrophages showed a dose response below and above a LaCl_3 concentration of 200 μM , while MG-63 and HBDC tolerated three times higher concentrations without reduction of viability. The viability of cells cultured on disks of the materials showed no differences between the reference and the lanthanum containing alloy. We therefore propose that lanthanum containing alloy appears to be a good alternative for biomedical applications, where machining of parts is necessary.

Keywords

Titanium alloy, lanthanum, machinability, corrosion, cytocompatibility, biomaterial, biomedical application

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Introduction

Titanium alloys like Ti6Al4V are widely used in the field of medical engineering which is based on the combination of low density, high strength and excellent biocompatibility. However, machining of titanium alloys for medical components, especially in deep hole drilling processes, involves high production costs because of their poor machinability ¹. Therefore, Lanthanum was added to the Ti6Al4V alloy to improve its machinability by strongly reducing the chip length. These results will be published elsewhere.

The combination of titanium and lanthanum is widely used, e.g. for the production of photocatalysts ^{2,3}. In contrast to other rare earth elements, which strengthen titanium and its alloys, lanthanum leads to strong embrittlement ⁴. In the biological environment lanthanum is well studied ⁵, the anticancerogenic properties of lanthanum chloride were described ^{6,7}, and it is administered as phosphate binding agent in chronic renal failure. Therefore we assume that the addition of lanthanum to an established titanium alloy like Ti6Al4V ⁸ should not lead to cytotoxicity.

In this paper the production route, the microstructure and the properties of the free machining titanium alloy Ti6Al4VLa0.9 are described. Additionally, the in vitro cytocompatibility which is of crucial importance for the use of these materials in medical applications as implants or surgical instruments was studied in detail. The cellular reaction of macrophages and bone cells with respect to elemental La³⁺ and the alloys was analysed.

Material and Methods

Preparation of titanium alloys and samples

To improve the machinability of Ti6Al4V, 0.9% of the rare earth metal element lanthanum were added to the alloy Ti6Al4V⁹. Ti6Al4V ELI (ASTM Grade 23, HWH – Hanseatische Handelswaren Gesellschaft, Bremen, Germany) and pure Lanthanum (purity 99.9%; Sigma Aldrich, Germany) served as starting materials. The alloy was fabricated by plasma arc melting, followed by casting the material into a water cooled copper crucible (fast cooling). The alloy was then deformed by rod extrusion at temperatures of 1050°C; ageing was possible up to 950°C to produce a recrystallised structure.

Standard compression test samples (diameter 11 mm, height 18 mm) were prepared for the hard X-ray experiments. The analysis of the samples was performed in the as cast state, the deformed state (end height after deformation 7 mm, degree of deformation $\phi \approx 0.9$) and in the deformed and aged state. Similar samples (diameter 11mm and height 18mm in the as cast state) were used to analyse the resistance to corrosion. Cylindrical specimens of 20 mm diameter and 10 mm thickness were machined for the electrochemical corrosion testing. The two flat cylinder surfaces were grinded with 1200 grid SiC paper and cleaned in ethanol prior to corrosion tests. For an estimation of the mechanical properties, standard round tensile test specimens (diameter 6 mm) were manufactured.

For the analysis of cytocompatibility of the materials, disks with a diameter of 10 mm and a height of 2 mm were prepared of Ti6Al4V (as reference) and Ti6Al4V0.9La. The disks were manufactured by cutting with a low speed diamond saw and a following surface treatment by electro polishing using phosphoric acid. Prior to cell experiments the disks were cleaned for 30 minutes each in acetone, chloroform

(VWR International, Darmstadt, Germany), a 2% Hellmanex-solution (Hellma GmbH & Co KG, Mühlheim, Germany) and distilled water. After cleaning, the specimens were dried at 37°C and steam sterilized.

Analysis of microstructure

The analysis of the lanthanum containing alloy microstructure was done by optical microscopy (Olympus PMG-3; Olympus, Hamburg, Germany) and scanning electron microscopy (SEM) including energy dispersive X-ray spectroscopy (EDS; LEO 1550, Zeiss, Oberkochen, Germany) after grinding (SiC disks, graining 240, 400, 800, 1200 and 2500 according to ASTM), polishing (OPS suspension and water) and etching (10 s in a solution of HF + H₂O₂; Merck, Darmstadt, Germany). Additionally, hard X-ray diffraction in Debye-Scherrer-configuration with a position sensitive detector at HASYLAB beam line BW5, 72.07 keV, $\lambda=0.0161$ nm, was chosen to perform phase analyses of the bulk material. Samples of 7 mm thickness in different stages (as cast, deformed, recrystallised) were investigated in transmission.

Mechanical testing

The mechanical properties were investigated by standard room temperature tensile tests and LCF-tests (8 Hz, 5×10^6 cycles, 8 samples per stress value). Standard round specimens with a diameter of 6mm were tested on a universal electro-mechanical testing machine (Zwick, Ulm, Germany). The values for force and elongation (50 values per second) were monitored during the material testing experiments. To ensure sufficient statistics, three samples were tested for each material state; minimum values are given in table 1.

Corrosion analysis

Immersion testing

Corrosion resistance of Ti6Al4V and Ti6Al4V0.9La alloys was analysed by exposure to different media (1.5% NaCl, acetic acid (50%) and aqua regia 120°C Merck, Darmstadt, Germany) for 24 hours under shaking conditions (according to DIN 38414 S4). Afterwards the mass of the sample was measured and the eluates were analysed by inductively coupled plasma optical emission spectroscopy (ICP-OES; Jobin Yvon JY 70, HORIBA, Munich, Germany). The analysis of the eluates included the elements titanium (Ti), aluminium (Al), vanadium (V) and lanthanum (La). Average values are given in table 2.

Electrochemical testing

The electrochemical tests were performed in 1.5 % NaCl at room temperature on flat cylindrical surfaces (perpendicular to the extrusion direction) using an ACM Gill AC potentiostat (ACM Instruments, Cumbria, UK). A sequence of tests, starting with 30 min measurement of the rest potential, followed by a single potentiodynamic polarisation and 22 electrochemical impedance spectroscopy measurements (EIS) with 1 h rest between each measurement was used. The electrolyte was not stirred during the experiments and saturated with atmospheric oxygen.

A three electrode set-up with an Ag/AgCl reference and a platinum counter electrode was used. After 30 min recording of the free corrosion potential, the polarisation scan was started from -200 mV relative to the free corrosion potential with a scan rate of 0.2 mV/s. The test was terminated when a corrosion current density of 0.1 mA/cm² was exceeded. From the cathodic branch of the polarisation curve the corrosion rate was determined using the Tafel slope. The low terminating limit was chosen to minimise the corrosion attack on the surface before the long term EIS measurements were started. The EIS measurements were carried out over a frequency range from

30 kHz to 0.1 Hz. The amplitude of the sinusoidal signals was 10 mV. The resistance values (charge transfer resistance) obtained at 0° phase shift (extrapolated using a simple Randles circuit model) were used as an indicator of the corrosion resistance of the specimens.

Isolation and culture of cells

Human bone derived cells (HBDC)

HBDC were grown out of bone chips obtained from patients undergoing total hip arthroplasty after the protocol of Gallagher ¹⁰. In brief, cancellous bone was cut into pieces of about 5 mm, and cultured in Dulbecco’s modified eagle medium (DMEM) Glutamax-I (Invitrogen Corporation, Karlsruhe, Germany) with 10% fetal bovine serum (FBS, PAA Laboratories GmbH, Linz, Austria), 1% penicillin and 100 µg/ml streptomycin (Invitrogen Corporation, Karlsruhe, Germany) for about ten days without medium change. At visibility of outgrowing HBDC, medium was changed every three days. Passaging was done at 70 – 80% confluence. Experiments were performed with cells in the 1st passage.

MG-63 cells

The human osteosarcoma cell line MG-63 was obtained from the european collection of cell cultures (ECACC, Salisbury, UK). The cells were cultured in the same medium as HBDC. Cells were passaged at subconfluency (70 – 80%) and reseeded in a density of 2 x 10⁴ cells/cm². For cell culture experiments cells in the 5th passage were used.

RAW 264.7 Cells

These tumour-derived mouse macrophages are capable of producing cytokines like nitric oxide (NO) and tumor necrosis factor α (TNF-α) as an in vitro immune response. Cells were cultured in DMEM low glucose with 2 mM glutamine and 10%

FBS and passaged at 60-70% confluence. Cells from the 5th passage were used for experiments.

Cytotoxicity assay

To evaluate a possible cytotoxic effect of elemental lanthanum, a cytotoxicity assay was performed. A 1 M solution of lanthanum chloride (LaCl₃; Sigma-Aldrich Chemie, Taufkirchen, Germany) was prepared. RAW 264.7, MG-63 or human bone derived cells were seeded in 96-well plates (Nunc, Wiesbaden, Germany) in a density of 5 x 10² per well/100 µL (2 x 10³ per well/100 µL for HBDC due to slower growth) and incubated at 37°C for 24 hours to allow cell adhesion. Thereafter, different concentrations of LaCl₃ were added to the wells. After another 24 hours incubation time, cell viability was measured by MTT. For each concentration 16 replicates were performed. LPS (10 ng/mL) served as positive control for the RAW cell line, tissue culture plastic (TCP) was used as standard. Experiments were repeated at least three times.

Macrophage response to material

To determine a possible inflammatory response caused by the applied materials the macrophage reaction of the mouse cell line RAW 264.7 was tested. 5x10⁴ per 30 µL macrophages were applied to the material samples and incubated at 37°C in an atmosphere of 5% CO₂ and 95% humidity for 48 h in 12-well cell culture plates (Nunc, Wiesbaden, Germany) covered with agarose to avoid unspecific cell adhesion. As a positive control lipopolysaccharide (LPS, 10 ng/mL; Sigma-Aldrich Chemie, Taufkirchen, Germany) was chosen. This bacterial endotoxin is a potent inducer of nitric oxide synthase (NOS-2)¹¹ and TNF-α¹² production. Cell viability was measured by methylthiazolyldiphenyl-tetrazolium bromide (MTT) and immune response by the content of tumor necrosis factor α (TNF-α) and nitric oxide (NO) in the medium.

Viability of bone-derived cells on the materials

As a measure for cellular reaction to the applied materials the MTT-assay was used. 5×10^4 MG-63 cells per 30 μ L or 1×10^4 HBDC per 30 μ L were applied to the material samples and incubated at 37° for two days in 12-well cell culture plates covered with agarose to avoid unspecific cell adhesion. Tissue culture plastic served as control. Thereafter, the MTT-assay was performed as described below.

Biochemical analysis

MTT-assay for metabolic activity

Viability was analysed by the cell proliferation kit MTT (Roche Diagnostics GmbH, Mannheim, Germany). The MTT-assay is based on the cleavage of the yellow tetrazolium salt MTT (methylthiazolyldiphenyl-tetrazolium bromide) into purple formazan by metabolically active cells. In brief, cells were cultured for two days on the carriers in agarose-coated 12-well plates with 2 mL of the cell-type specific medium. 1 mL of the supernatant was discarded and 100 μ L of the MTT-solution (5 mg/mL MTT in phosphate buffered saline PBS) was added. After an incubation period of 5 hours the cells were lysed and the formazan crystals solubilized by adding 1 mL solubilization solution (10% SDS in 0.01M HCl; Merck, Darmstadt, Germany) and incubation overnight in a humidified atmosphere (37° C, 5% CO₂). The solubilized formazan product was photometrically quantified using an ELISA reader (Tecan Sunrise, TECAN Deutschland GmbH, Crailsheim, Germany) at 570 nm with a reference wavelength of 655 nm.

The protocol was adjusted for the examinations of elemental lanthanum, which were performed in 96-well plates. Here, the cells were incubated for 24 hours with 100 μ L medium and different concentrations of LaCl₃, afterwards 10 μ L of the MTT-solution and 100 μ L of solubilization solution were added.

Tumor necrosis factor α (TNF- α) determination

Levels of TNF- α were measured with a sandwich enzyme-linked immunosorbent assay (ELISA) according to the manufacturers' instructions (R&D Systems, Wiesbaden, Germany). Briefly, 50 μ L of standard, control, or sample were added per well in a 96-well plate. The samples were incubated for 2 h at room temperature, followed by two rinses in washing buffer to remove unbound substances. Then, 100 μ L of an enzyme-linked polyclonal antibody were added to each well and incubated for 2 h at room temperature. After a second washing step, 100 μ L of substrate solution was added to each well and then incubated in the dark for 30 min. The optical density of each well was determined using an ELISA reader at 450 nm with a reference wavelength of 570 nm. The sample concentrations were determined by comparison with a standard curve. All samples were assayed in triplicate.

Nitric oxide (NO)-measurement

The production of nitric oxide by macrophages is induced by stimulators of the immune response, e.g. LPS. The amount of nitric oxide was assessed by the measurement of NO₂ in the medium. An aliquot of the medium (100 μ L) was mixed with 50 μ L of Griess reagent (Fluka, Taufkirchen, Germany) and incubated for 5 minutes at room temperature. Then the absorbance at 542 nm was determined on an ELISA-reader. Solutions of sodium nitrite (NaNO₂) (Sigma Aldrich Chemie, Taufkirchen, Germany) diluted in DMEM served as standards.

Statistics

Statistics were performed using the SigmaStat package (Systat software GmbH, Erkrath, Germany). Standard analysis comparing more than two treatments was done by using the One-way ANOVA. Depending on the data distribution either a one-way ANOVA or an ANOVA on ranks was performed. Post hoc tests were Holm-Sidak

or Dunn’s versus the control group, respectively. Statistical values are indicated at the relevant experiments.

Results

Material properties

As expected, after casting Ti6Al4V0.9La a martensitic α' titanium matrix with dissolved aluminium and vanadium developed, containing discrete round particles with a high lanthanum content mainly on the grain boundaries (Fig. 1). The average particle size was about 3 μm . In hard X-ray diffraction no lanthanum oxide was found, whereas metallic lanthanum (α -La and β -La) was identified (Fig. 2).

The mechanical properties were investigated by room temperature tensile tests (table 1). It is obvious that the tensile strength of the lanthanum containing alloy was decreased compared to the standard alloy. Additionally, toughness was reduced. Nevertheless, the requirements of ASTM 348 for Ti6Al4V are still fulfilled by Ti6Al4V0.9La in the recrystallised state. The fatigue limit was determined to be 480MPa.

Corrosion

The results of the corrosion experiments are shown in table 2. The corrosion resistance of the lanthanum containing alloys were similar in NaCl and acetic acid compared to the standard alloy. The mass reduction of the test samples was less than 0.1%. In aqua regia the eluates of Ti6Al4V0.9La contained higher concentrations of all elements investigated. The reduction in mass was about 0.2% for Ti6Al4V and 0.8% in Ti6Al4V0.9La.

Additionally, the corrosion rate was determined by short-time polarisation measurement. The Ti6Al4V material showed a very low corrosion rate of 4.4×10^{-4} mm/year. The addition of lanthanum to this alloy increased the corrosion rate by a factor of ten (corrosion rate Ti6Al4V0.9La: 3.1×10^{-3} mm/year). Both alloys remained passive during the potential scan of up to 2200 mV, while the TiAl6V4 alloy had the slightly lower passive current density (Fig. 3). The average charge transfer resistance values of $1.107 \times 10^6 \Omega\text{cm}^2$ (Ti6Al4V0.9La) and $1.603 \times 10^6 \Omega\text{cm}^2$ (Ti6Al4V) determined from the EIS measurements corresponded well with the other corrosion performance, especially the passive current densities.

Cellular reaction to elemental lanthanum

The corrosion measurements showed that lanthanum was detectable in NaCl-solution demanding the investigation of cellular reaction to elemental lanthanum. The viability of cells which were exposed to different concentrations of lanthanum was analysed using three cell types. Macrophages were chosen as they are the first cells to get in contact with implant surfaces (Fig. 4 a). A concentration dependent decrease in cell viability was observed at concentrations between 20 and 80 μM . The following increase in viability peaked at a concentration of 200 μM , where viability was in the range of the control (cells cultured on tissue culture plastic). The following sharp decrease resulted in nearly no viable cells at concentrations above 500 μM LaCl_3 . Statistically significant differences compared to the control were observed for all values except 10, 20 200 and 300 μM (ANOVA on ranks; $p < 0.05$).

To evaluate the response of bone cells osteoblast-like cells (MG-63, Fig 4 b) and HBDC (Fig. 4 c) were used. Both cell types represent relevant species for implant contact, the MG-63 as an 'artificial' cell line and HBDC as primary cells. In contrast to the results obtained using the macrophage cell line, the addition of lanthanum had no

effects on cell viability below a concentration of 100 μM . At this concentration the highest viability was observed for both cell types (MG-63: 116 % of control, HBDC: 101 %). MG-63 showed a decrease in viability at higher concentrations and only low viability at 2 mM LaCl_3 , while HBDC also tolerated concentrations of 2 mM. In general, cell viability of HBDC was slightly reduced compared to the control in the range of about 10 %. It was significantly decreased compared to the control at 10, 20, 70, 90 and 800-1000 μM , with values ranging from 74 % to 89 % (ANOVA on ranks, $p < 0.05$). In contrast, the values measured for MG-63 were significantly increased compared to the control between 20 and 50 μM , and the decrease in viability became significant at a concentration of 700 μM LaCl_3 .

Cell interaction with the alloys

Macrophage response

RAW 264.7 cells were seeded as described and cell viability was measured after an adhesion time of 48 hours. Concerning the viability on the two materials no significant differences were observable (Mann-Whitney Rank Sum test, $n=37$, $p=0.42$), a cytotoxic effect of the materials was not determined (Fig. 5). The release of $\text{TNF-}\alpha$ into the medium after 48 h was increased by a factor of 7 around the titanium alloys compared to tissue culture plastic. Between the two alloys no significant differences were observable, whereas the treatment with LPS induced a significant increase (18-fold compared to the control and 2.5 fold compared to the materials) in $\text{TNF-}\alpha$ production (Fig. 8; one-way ANOVA, $n=8$ per group). Nitric oxide was not detected, except for LPS ($1.4 \pm 0.26 \mu\text{g/mL}$).

Reaction of bone like cells

For the determination of the reaction of bone-derived cells two different cell types were used: 1. the osteosarcoma cell line MG-63 and 2. cells derived from human bone. Values were calculated as percent of viable cells cultured on tissue culture plastic (control), determined by MTT. In both cases the viability showed no significant differences between the two materials (MG-63: Mann-Whitney rank sum test; $n=35$, $p=0.677$; HBDC: Mann-Whitney rank sum test, $n=17$, $p=0.182$). Hence, for bone-like cells no hint for materials cytotoxicity is evident (Fig. 6).

Discussion

The aim of our study was to investigate whether by the addition of lanthanum to a base titanium alloy the machinability can be improved while biocompatible properties comparable to the widely used Ti6Al4V alloy⁸ remain.

The alloy Ti6Al4V0.9La exhibited a martensitic α -titanium matrix with metallic lanthanum mainly in the grain boundaries leading to reduced strength and elongation compared to the Ti6Al4V alloy. In former studies rare earth metals (REM), namely erbium, lanthanum and cerium have been used to improve the mechanical properties of titanium alloys using particle strengthening by REM oxides^{4,13,14}. These alloys were never introduced into industrial application because of oxidation and strong embrittlement of the rare earth metals in such materials. In our studies moderate cooling rates have been used as bulk specimen of the material were cast into a copper mould. Therefore metallic lanthanum particles developed in the micro scale. Even after long annealing times (e.g. 1050°C / 3h / FC) no lanthanum oxide particles were formed. Hence, the fracture toughness of Ti6Al4V0.9La was slightly reduced due to soft particles on the grain boundaries but still sufficient for applications in biomedical engineering.

Titanium alloys are highly corrosion resistant when the surface is covered by a passivating oxide layer. If this layer is broken down, ions from the material are released into solution ^{15,16}. The release is stopped, when the passive layer is regenerated by self-healing ¹⁷. Here we showed that the regeneration of the oxide layer **was** slower in the Ti6Al4V0.9La material. A reduction in corrosion resistance was observed in boiling aqua regia, where all elements were released from the Ti6Al4V0.9La material in higher concentrations and the mass reduction was increased from 0.2% (measured for the base alloy) to 0.8%. This was also confirmed by the short-time polarisation measurements, where the Ti6Al4V0.9La showed a higher passive current density than the base alloy. This result seems to be a contradiction to the praxis of lanthanum addition to increase the corrosion resistance of aluminium alloys. But this effect was not observed here because the corrosion protective $\text{La}(\text{OH})_3$ forms only at alkaline $\text{pH} < 9$ ¹⁸, which **was** not reached in our corrosion studies. However, a release of lanthanum from the material may have a beneficial effect for adhering cells, as La^{3+} has the ability to reduce reactive oxygen species (ROS, ¹⁹). The Ti6Al4V alloy has recently been reported to induce permanent oxidative stress in endothelial cells ²⁰. Hence, the addition of an antioxidant alloy element may reduce this not yet understood phenomenon. Additionally, corrosion behaviour is altered in the presence of proteins ²¹. This will be examined in ongoing studies.

Lanthanum is administered as a phosphate binder in chronic renal failure as lanthanum carbonate. In the course of the clinical trials many studies were performed which showed no toxicity, although lanthanum accumulated in different organs ^{22,23}. With respect to bone Bervoets et al. showed an osteomalacia in lanthanum carbonate treated rats in combination with an accumulation of lanthanum in the bone ²⁴. The number of osteoblasts was not altered and the lesions in the bone

disappeared after a wash out period of 8 weeks. Therefore they concluded that the high phosphate binding capacity of LaCO_3 leading to phosphate depletion is likely to be the reason for the altered bone metabolism, not the lanthanum itself ²⁴. This is supported by the reaction of MG-63 and human bone derived cells to elemental lanthanum. They tolerated concentrations up to 600 μM without significant decrease in cell viability, and at higher concentrations up to 1 mM still 80% of the cells survived. The loss of MG-63 viability at 2 mM LaCl_3 is probably due to their origin as cancer derived cells. LaCl_3 administration on leukemic cell lines reduced their viability and induced apoptosis, while mesenchymal stem cells were unaffected ⁶. This was also observed comparing HBDC and MG-63. Although both cell types followed the same trend, HBDC also tolerated higher concentrations. In contrast, the macrophage cell line RAW 264.7 showed a dose response already at low concentrations, followed by a maximum at 200 μM LaCl_3 , thereafter a sharp decline in viability was found. This effect was also observed in other studies, where an exposition of macrophages to lanthanum/zinc for more than 60 minutes induced progressive loss of membrane integrity, measured by trypan blue exclusion assay ²⁵. Lanthanum in the used concentrations has an impact on the lateral compression pressure of membranes ²⁶⁻²⁸, and it has been described as an inducer of apoptosis ²⁹. Additionally, recently an amplification of apoptosis by membrane-derived microparticles was discovered, which were observed in different macrophage cell lines ^{30,31}. The lanthanum exposition time in our study was high (24 hours), and the reduction of cell viability may be explained by the induction of apoptosis by direct action on the cell membrane of macrophages. This will be the focus of further studies.

In spite of these negative effects on macrophages, the observed release of lanthanum from the material using NaCl as corrosive medium was in the range of 11 nM. The concentrations used for the cytotoxicity assay started at a concentration of

10 μM , a 1000-fold higher concentration, while significant cellular reactions were observed at 200 μM , a further 20-fold increase. Lanthanum which is directly released from the material due to corrosion therefore should not induce negative effects. Hence, in the experiments performed with direct contact between cells and materials, neither cytotoxic effects of the new alloy were observed, nor differences in cell viability between Ti6Al4V and Ti6Al4V0.9La. In another study dealing with lanthanum-containing hydroxyapatite ³², the authors did not find an increase in the materials toxicity due to added lanthanum. However, macrophages react to the material with elevated values of TNF- α , a proinflammatory cytokine, while nitric oxide, another proinflammatory molecule, was not produced. An upregulation of TNF- α production of macrophages in contact with titanium was also observed in other studies ^{33,34}. Although titanium is claimed to be an inert material, evidence is rising, that it is thrombogenic and activates the complement system, which again is inducing cytokine secretion. Aluminum also was shown to be an activator of the complement sytem ³⁵.

Conclusion

The addition of lanthanum led to an alloy with improved machinability. The amount of lanthanum directly influenced the mechanical properties which are still sufficient for many applications especially for tools in medical engineering. The cytocompatibility of the material was comparable to that of Ti6Al4V; therefore the alloy may be a good alternative for serial production. As the solvability of lanthanum in α -titanium is extremely small at room temperature, this approach can most likely be transferred to other near- α or α - β -alloys, namely Ti6Al7Nb, which is nowadays introduced into

applications in biomedical engineering. Future work will therefore focus on the improvement of machinability of the Ti6Al7Nb alloy.

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Figure Captions

Figure 1: Microstructure of the alloy Ti6Al4V0.9La after melting and casting. Discrete particles precipitate in the titanium matrix. SEM-image in backscattering mode (detector RDSD, 15keV).

Figure 2: Hard (72 keV) X-ray analyses of the alloys Ti6Al4V and Ti6Al4V0.9La. No lanthanum oxide can be found, whereas cubic lanthanum can be easily identified in Ti6Al4V0.9La. The strong peaks refer to α -titanium.

Figure 3: Comparison of the potentiodynamic polarisation scans determined for pure Ti6Al4V and Ti6Al4V alloyed with lanthanum in 1.5% NaCl solution at pH 6.5.

Figure 4: Cell viability of (A) RAW 264.7 mouse macrophages, (B) MG-63 human osteosarcoma cells and (c) HBDC exposed to different concentrations of lanthanum chloride (LaCl_3). The asterisks indicate significant differences in cell viability (ANOVA, * = $p < 0.05$).

Figure 5: TNF- α release into the medium by RAW 264.7 cells around the material samples ($n=8$). TCP served as negative control, lipopolysaccharide (LPS) as positive control. The increase in TNF- α release induced by LPS compared to the control is significant (One way ANOVA on ranks, $p < 0.05$). Between the two titanium alloys no significant differences were determined.

Figure 6: MTT-values calculated as percent of control for RAW 264.7 mouse macrophages (RAW, black bars), human bone derived cells (HBDC, white bars) and

MG-63 cells (grey bars) cultured for 48h on Ti6Al4V and Ti6Al4V0.9La disks. Bars represent mean values + standard deviation. No significant differences in cell viability were found for RAW 264.7, MG-63 and HBDC.

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Table 1: Mechanical properties of Ti6Al4V and Ti6Al4V0.9La

Alloy	State	Strength $R_{p0.2}$ / N/mm ²	Elongation / %
Ti 6Al4V ELI	recrystallised	960	21
Ti6Al4V0.9La	as cast	620	1
Ti6Al4V0.9La	extruded	950	7
Ti6Al4V0.9La	recrystallised	860	11

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Table 2: Results of corrosion tests on Ti6Al4V and Ti6Al4V0.9La. Analysis of the eluates by ICP-OES. Values are given in ppm and μMol (in brackets), DL: limit of detection.

Material	Ti6Al4V			Ti6Al4V0.9La			
Element	Ti	Al	V	Ti	Al	V	La
NaCl (1.5%)	<DL	<DL	<DL	<DL	<DL	<DL	1.5 (0.011)
CH ₃ COOH (50%)	7.9 (0.165)	1.3 (0.048)	0.5 (0.0098)	7.9 (0.165)	1.5 (0.055)	0.5 (0.0098)	5.5 (0.037)
Aqua Regia (120°C)	332 (6.934)	22.3 (0.826)	16.3 (0.32)	536 (11.195)	39.2 (1.453)	27.6 (0.542)	20.7 (0.149)

130x97mm (200 x 200 DPI)

182x121mm (600 x 600 DPI)

220x165mm (600 x 600 DPI)

204x324mm (600 x 600 DPI)

134x104mm (600 x 600 DPI)

133x99mm (600 x 600 DPI)