Examination of a biodegradable magnesium screw for the reconstruction of the anterior cruciate ligament: A pilot in vivo study in rabbits

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ARTICLE INFO

Article history:
Received 22 May 2015
Received in revised form 28 October 2015
Accepted 13 November 2015
Available online 14 November 2015

Keywords:
Magnesium alloy
Interference screw
Biodegradable
Rabbit
Biocompatibility
Tendon

ABSTRACT

The reconstruction of the anterior cruciate ligament is, for the most part, currently performed with interference screws made of titanium or degradable polymers. The aim of this study was to investigate the use of biodegradable magnesium interference screws for such a procedure because of their known biocompatibility and reported osteoconductive effects. The left tibiae of each of 18 rabbits were implanted with a magnesium-based (MgYREZr-alloy) screw, and another 18 with a titanium-based control. Each group was divided into observation periods of 4, 12 and 24 weeks. After sacrifice, μCT scans were acquired to assess the amount of the gas liberated and the degradation rate of the implant. Histological evaluations were performed to investigate the local tissue response adjacent to the implant and to assess the status of the attachment between the tendon and the bone tissue. The μCT scans showed that liberation of gas was most prominent 4 weeks after implantation and was significantly decreased by 24 weeks. All screws remained in situ and formed a sufficient connection with the tendon and sufficient osseous integration at 24 weeks. Histological evaluations showed neither inflammatory reactions nor necrosis of the tendon. The results of this pilot study in rabbits indicate that this magnesium-based interference screw should be considered as an alternative to conventional implant materials.

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1. Introduction

Arthroscopic reconstruction is considered the gold standard nowadays for repairing a traumatically ruptured anterior cruciate ligament (ACL). The aim of such reconstruction is to provide the knee with its original level of stability and in this way avoid the development of osteoarthritis [1]. In active patients e.g. athletes, conservative treatments often fail. There are autograft and allograft options, and autografts such as quadrupled hamstring tendons and bone–patellar tendon–bone transplants are commonly used [2,3]. In ACL reconstruction surgeries, grafts are typically fixed to their targets using either bioreabsorbable or metallic screws. Metal interference screws were used in the early days of such surgeries [4], followed by the use of bioreabsorbable screws in the early 1990s [5]. The main component in the most commonly used bioreabsorbable screws is polylactic acid. Many other types of different bioreabsorbable screws are also available, e.g., those with polyglycolic acid, polypropyaxodanone, poly-L-lactic acid and poly-D-lactic acid.

Metallic and bioreabsorbable interference screws have shown comparable biomechanical results [6,7], even though several negative effects such as tunnel widening, implant breakage, implant dislocation and foreign body reactions have been described [8–11].

Several magnesium alloys have recently been investigated for use in orthopedics [12]. These alloys can, for example, reduce stress shielding, due to their biomechanical properties (elastic modulus and compressive yield strength) being comparable to those of natural bone [13,14]. Magnesium alloys also provide osteoconductive effects [15]. A rabbit model study published by our working group last year showed no degradation-provoked negative effects on the synovial membrane of the knee joint when the magnesium alloy MgYREZr was used than when a titanium alloy was used, suggesting that this magnesium alloy could be suitable for intra-articular use [16]. In other studies, MgYREZr has shown good in vivo results and host response in osseous environments [17,18]. We also recently showed in an in vitro study that the initial extent of dislocation of a MgYREZr-based interference screw is less, and its maximum load before failure is greater, than those properties of a commercial polymer screw [19].

ACL reconstruction surgery may be a novel area of application for magnesium-based implants, but little is known about the magnesium-
ligament interface and about the effects of the degradation products of the magnesium alloy (and the resulting gas formation) on the integration of the ligament in the bone. The primary stability of the ligament in ACL reconstruction surgery during the first 12 weeks is crucial; therefore, the mechanical integrity of the attachment of the ligament to the bone should be made as high as possible. During this time period, the attachment should be disturbed as little as possible. In the present pilot study, we investigated the effects of a degradable MgYREZr-based screw on a fixed ligament in rabbit tibia in vivo. A non-degradable titanium screw was used as a control in this approach.

2. Materials and methods

2.1. Study design

The animal experiment was conducted using a protocol approved by an ethics committee of the local authorities Lower Saxony State Office for Consumer Protection and Food Safety (LAVES), in accordance with German federal animal welfare legislation (Approval No. 33.9-42502-04-12/0976) and with the National Institute of Health guidelines for the use of laboratory animals. Thirty-six female New Zealand white rabbits (Charles River, Bad Kissingen, Germany), with a mean body weight of 3.8 ± 0.2 kg at an age of 6 months, were randomized into two implant groups of 18 animals each. Interference screws made of MgYREZr were implanted in the first group, and identically constructed screws made of the titanium alloy Ti6Al4V in the second group. Each group was subdivided into three subgroups with implantation periods of 4, 12 and 24 weeks, with 6 animals in each subgroup.

2.2. Implants

In the present study, twist-off screws made of MgYREZr (obtained from Syntellix AG, Hannover, Germany) and of Ti6Al4V (obtained from Königssee Implantate, Allendorf, Germany) were utilized. The magnesium alloy is from the MgYREZr alloy system according to DIN EN 1753 which is similar to WE43: it has a magnesium fraction of over 90% and a particle size of less than 10 μm, and was manufactured using a powder metallurgical process. Due to its microstructure, this magnesium alloy exceeds a yield strength of >260 MPa, a tensile strength of >290 MPa, and an elongation to failure of >8%. The screws were produced in the Institute of Production Engineering and Machine Tools (IFW) of the Leibniz Universität Hannover, Hannover, Germany. The implants had a total length of 10 mm and an external diameter of 2.6 mm with a thread pitch of 0.8 mm (Fig. 1). The magnesium and titanium screws were manufactured from extruded bars (6 mm or 12 mm, respectively) on a computerized numerically controlled (CNC) lathe and a CNC milling machine in dry cutting processes. Every implant had a hexagonal screw head (5 × 5 mm) for insertion. All screws were ultrasonically cleaned in ethanol for 10 min. The screws were not further processed or coated. All samples were gamma sterilized, with 25 kGy of cobalt-60 γ-radiation (BBF Sterilization Services, Kernen, Germany), prior to implantation.

2.3. Surgical method

Surgery was performed under general anesthesia, induced by intramuscular injection with ketamine (25 mg/kg; Ketanest, Albrecht, Aulendorf, Germany) and midazolam (5 mg/animal; Dormicum, CuraMED Pharma, Karlsruhe, Germany), and a subcutaneous dose of glycopyrrolate bromide (0.1 mg/animal; Robinul, Riemser Arzneimittel, Greifswald — Insel Riems, Germany). For analgesia, the rabbits were given meloxicam (0.15 mg/kg; peroral; Metacam, Boehringer Ingelheim, Germany) one day preoperatively, immediately preoperatively, and for the following two days. Additionally, they received preoperative buprenorphine (0.15 mg/animal; s.c.; Temgesic, Boehringer Ingelheim, Germany), and postoperative ketoprofen (1.0 mg/kg; injectable; Ketoprofen, FMS, Jena, Germany), and a subcutaneous dose of glycopyrrolate bromide (0.1 mg/animal; Robinul, Riemser Arzneimittel, Greifswald — Insel Riems, Germany) one day postoperatively. For analgesia, the rabbits were given meloxicam (0.15 mg/kg; peroral; Metacam, Boehringer Ingelheim, Germany) one day preoperatively, immediately preoperatively, and for the following two days. Additionally, they received preoperative buprenorphine (0.15 mg/animal; s.c.; Temgesic, Boehringer Ingelheim, Germany), and postoperative ketoprofen (1.0 mg/kg; injectable; Ketoprofen, FMS, Jena, Germany), and a subcutaneous dose of glycopyrrolate bromide (0.1 mg/animal; Robinul, Riemser Arzneimittel, Greifswald — Insel Riems, Germany) one day postoperatively.

Fig. 2. Schematic representation of the operation technique. In the left stifle joint of each rabbit, a lateral parapatellar arthrotomy was performed to gain access to the femoral notch. The tendon of the extensor digitorum longus muscle was released from the lateral femoral condyle. Afterwards, a hole was drilled perpendicular to the long axis of the tibia using a manually operated drill with a diameter of 2.7 mm. The tendon was passed through the tunnel drilled in the bone and fixed with an interference screw.
Especially for their general conditions, possible gas cavities, postoperatively, and were observed daily by an experienced veterinarian.

2.5. Postoperative treatment and radiological evaluation

The rabbits were allowed unrestricted movement in their cages postoperatively, and were observed daily by an experienced veterinarian for their general conditions, possible gas cavities, inflammation, joint effusion and lameness. Antibiotic medication was given before surgery and continued for four more days (Enrofloxacin, 10 mg/kg, Baytril 2.5%, p.o., once daily, Bayer Animal Health, Leverkusen, Germany). Radiographs (anterior-posterior and lateral views) were taken immediately after surgery and after euthanasia to verify the implant position and to detect possible formation of gas cavities.

2.4. ICP-MS analysis of alloying elements in blood

Blood samples were taken before surgery (n = 2) and at the end of the implantation periods (n = 6 per implantation time) to monitor possible elevated levels of alloying elements in the blood. The blood samples were analyzed in a diagnostic laboratory (Medizinisches Labor Waldbronn, Germany). Inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500ce, Agilent Technologies Deutschland, Waldbronn, Germany) was used to determine the concentrations of yttrium (Y) and zirconium (Zr). The samples were prepared according to a standard protocol [21]. The limit of quantification of Y was 0.05 μg L\(^{-1}\) and 0.07 μg L\(^{-1}\) for Zr. The reference values of these two alloying elements in human blood comprises a range of 0.042–0.308 μg L\(^{-1}\) for Y and 0.2–9.7 μg L\(^{-1}\) for Zr [22].

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2.6. Micro-computer tomography and determination of gas and implant volumes

After euthanasia of the rabbits, their bones were harvested and scanned by micro-computed tomography (μCT Siemens Inveon, Siemens Medical Solutions, Knoxville, USA). The μCT-scans of the samples were performed at a voltage of 80 kV, a current of 500 μA, an integration time of 800 ms and a resolution of 25.6 μm. Both the volumes of the degradable magnesium alloy implant and the gas cavities were determined by means of the software Inveon Research Workplace 4.2. All implants of the magnesium group were manually contoured to distinguish bone from the implant material, and a threshold of >600 Hounsfield units (HU) was set on the basis of scans from the implants before implantation. Five magnesium alloy implants were scanned prior to implantation for determination of the implant volume in its initial state. The average degradation rate in millimeters per annum (mm a\(^{-1}\)) was calculated according to the following equation of Witte et al. [23]:

\[
CR = 365 \Delta V / (At)
\]

In this calculation, CR (mm a\(^{-1}\)) is the degradation rate, ΔV (mm\(^3\)) the volume loss, A (mm\(^2\)) the area which was subjected to the corrosion, t (days) the implantation period, and 365 is the conversion factor to arrive at the unit mm a\(^{-1}\). For the evaluation of the accumulation of gas, a threshold of ~700 HU was applied for all scans. This value was chosen on the basis of HU values of bone that was scooped out.

**Fig. 3.** Radiological images of the left hind leg with an interference screw made of the magnesium alloy MgY2REZr (marked by white arrow heads) and the titanium alloy Ti6Al4V at 4, 12 and 24 weeks after implantation. Gas cavities appeared in the magnesium group (marked by small white arrows) at a) 4 and c) 12 weeks. The magnesium implant is still visible at e) 24 weeks.
2.7. Histology of soft tissues

2.7.1. General histology of organs (liver, kidneys and lymph nodes)

Liver, kidney and lymph node (lymphonodi poplitei) tissues were fixed in 3.5% commercial buffered formalin (Otto Fischar, Saarbrücken, Germany) for two days at room temperature, embedded in paraffin according to standard protocols, cut into 5-μm-thick sections using a RM 2155 microtome (Leica, Bensheim, Germany) and placed onto poly-L-lysine-coated glass slides. Before staining, the samples were deparaffinized in xylene (2 × 10 min) and rehydrated in a series of decreasing concentrations of alcohol.

2.7.2. Hematoxylin and eosin staining

Sections were first rinsed in distilled water for 30 min, then stained for 20 s with Mayer’s hematoxylin (Merck, Darmstadt, Germany), rinsed in tap water for 10 min, then stained with 1% eosin (Merck), dehydrated in a graded concentration series of ethanol and mounted in Eukitt (Labonord, Mönchengladbach, Germany) according to the standard procedure. The sections were evaluated histopathologically with regard to pathological alterations, inflammatory processes and presence of metallic particles in a certified veterinary diagnostic laboratory (Laboklin, Bad Kissingen, Germany).

2.8. Histology of hard tissues

After the μCT scans, the bone samples were fixed in commercial 3.5% formalin for 5 days at room temperature. The tissues were then subjected to embedding and polymerization in methyl methacrylate (Technovit 9100 New, Heraeus-Kulzer, Hanau, Germany) according to the manufacturer’s instructions and established protocols [24]. After polymerization, the tissue blocks were further processed using the cutting-grinding technique [25]. For this purpose, the blocks were cut with a special diamond band saw (Leica SP1600 saw microtome, Leica Biosystems, Nussloch, Germany) into sections of 500 μm thickness. These sections were then ground with a special plate grinder (Exakt 400CS, Exakt) to a thickness of 30–50 μm. The sections were classified as medial, intermediate, and lateral, according to their anatomical locations.

2.8.1. Intravital specific fluorochrome staining

Intravital fluorochrome staining was performed with subcutaneous injections of calcein green (Waldeck, Münster, Germany; 2% solution with sodium hydrogen carbonate, 10 mg/kg) at days 14 and 19 post surgery for animals in the 12- and 24-week groups and at days 7 and 12 post operation for the 4-week observation group. In all groups, xylenol orange (Sigma-Aldrich, Steinheim, Germany; 2% solution with sodium hydrogen carbonate, 90 mg/kg) was administered at nine and four days before euthanasia.

2.8.2. Toluidine blue staining

The sections were stained with the toluidine blue staining method. Sections were incubated in 0.1% toluidine blue O (Sigma, Taufkirchen, Germany) for 40 s, washed in distilled water, dehydrated in alcohol and mounted with Eukitt (Labonord, Mönchengladbach, Germany).

Fig. 4. Results of ICP-MS analysis of the alloying elements a) zirconium and b) yttrium in blood samples at 4, 12, and 24 weeks after implantation (n = 6). a) Elevated levels of zirconium were observed in the 4-week group. At 12 and 24 weeks, all values were within the reference range. b) Median levels of yttrium were within the reference range at all examination times.
2.8.3. Microscopy
Photomicrographs were taken with a Zeiss Axioskop 40 or a Zeiss Imager Z1 microscope equipped with epifluorescence and polarization optics and a scanning stage (Imager Z1). Both microscopes were combined with a Zeiss AxioCam Mrc digital camera and Zeiss AxioVision software (all from Zeiss, Oberkochen, Germany).

2.8.4. Analysis of the direct contact between bone and implant
First, the perimeter of the implant was determined using AxioVision software. Then, the length of the part of the bone closely attached to the surface of the implant was determined. In the case of the magnesium implants, the original surface of the implants was used. For this analysis, 1–2 sections were used per animal from each location (lateral, intermediate, and medial). For each implant location and implantation time, a minimum of 6 sections were examined.

2.8.5. Pathological examination of the bone-implant-interface
The pathological evaluation of the bone-implant-interface was carried out by a veterinary pathologist of the Fraunhofer-Institute for Toxicology and Experimental Medicine (Hannover, Germany). For every animal, two locations were assessed on the bone (lateral and medial).

2.9. Statistical analysis
The results of this study were analyzed using the R statistical package, version 2.15 (R Foundation for Statistical Computing, Vienna, Austria). The micro-CT data and element analysis in blood were analyzed according to the Wilcoxon test, which was also applied to measure the extent to which the differences between the bone-implant contacts of the magnesium and titanium groups were statistically significant. Results with p ≤ 0.05 were considered significant.

3. Results

3.1. Clinical observation showed stable fixation and good compatibility of both implant groups
Clinically, all implants were tolerated well without any signs of inflammation, lameness or evidence of subcutaneous gas cavities. The fixation of the tendon was stable, and no rupture or loosening was seen. The radiological outcome showed that the implants remained in situ and gas cavities appeared in the magnesium group at 4 and 12 weeks after implantation (Fig. 3).

3.2. Alloying elements were present in blood serum only in very low concentrations
The results of the inductively coupled plasma mass spectrometry (ICP-MS) analysis showed elevated levels of zirconium four weeks...
after implantation. At this time, a mean value of $1.53 \pm 0.7 \, \mu \text{g L}^{-1}$ zirconium was detected in the collected blood samples. This mean value decreased to $0.7 \pm 0.11 \, \mu \text{g L}^{-1}$ and $0.52 \pm 0.18 \, \mu \text{g L}^{-1}$ at 12 and 24 weeks after implantation, respectively, which were within the reference value for this element, but higher than values before implantation (Fig. 4). The yttrium and zirconium concentration in blood samples before implantation were below the level of quantification.

There was one elevated yttrium value in the blood samples 4 and 24 weeks after implantation. The mean values after the different implantation periods were also below the level of quantification before implantation (4 weeks: $0.18 \pm 0.08 \, \mu \text{g L}^{-1}$, 12 week: $0.11 \pm 0.02 \, \mu \text{g L}^{-1}$, 24 weeks: $0.17 \pm 0.11 \, \mu \text{g L}^{-1}$).

3.3. The gas volume significantly decreased by 24 weeks after implantation and a degradation rate was determined

μCT-scans of the bone implant samples were performed at 4, 12, and 24 weeks after implantation. The contours of the magnesium screws were visible at all of these investigation times. Gas accumulated in the medullary cavity, with the most gas observed 4 weeks after implantation (Fig. 5a). Both the magnesium and the titanium screws were well integrated in the cortical bone 24 weeks after implantation. The μCT images of the titanium screws showed the material specific artifacts on the edges of the implants (Fig 5b, d, e).

The original magnesium screws had a mean initial volume of $46.2 \pm 2.5 \, \text{mm}^3$ with a mean surface area of $114.6 \pm 5.4 \, \text{mm}^2$. The volumes of the magnesium screws decreased by approximately $25 \pm 11.5\%$ 4 weeks after implantation (Fig. 6a). The implant volumes determined 12 and 24 weeks after implantation did not significantly differ from the volume determined four weeks post-surgery. Within the investigated period a degradation rate of $0.17 \, \text{mm per year}$ could be determined for the magnesium screw taking the initial and the volume 24 weeks post-surgery into account.

A volume of $330.5 \pm 83 \, \text{mm}^3$ gas was detected in the medullar cavity in proximity of the implant 4 weeks after implantation (Fig. 6b). Significantly lower gas volumes were found 12 weeks after implantation, and the gas volume was lowest 24 weeks post-surgery ($12.9 \, \text{mm}^3$). A μCT 3D reconstruction of the implant and gas is given in Fig. 7.

3.4. Pathology of distinct organs demonstrated no alterations

The organs showed no pathological alterations and no signs of inflammation for both types of screws (Fig. 8). For one of the magnesium screws and one of the titanium screws, crystalline particles were found in macrophages.

3.5. Sufficient connection of bone and tendon tissue was determined in both groups after 24 weeks

Fig. 9 shows examples of histological sections of magnesium and titanium samples acquired at the indicated times after implantation. The pathological evaluations of these two groups demonstrated that there was no evidence of inflammatory reactions, fibrosis, or necrosis. There was only a focal infiltration of macrophages and granulocytes in the tendon tissue in one section of the magnesium group and two sections of the titanium group. Tendon tissue was observed in all sections of both groups at 4 weeks after implantation, and in some sections, at 12 and 24 weeks after implantation. Tendon tissue was visible on the histological sections and when analyzed with polarized microscopy (Fig. 10). In three sections from the magnesium implant group, no contact was detected between the tendon tissue and the bone tissue; instead, the tendon was unattached in the bone marrow. Additionally, in this group, some sections revealed that the tendon fibers were separated, and the spaces between fibers were filled with either gas (6 sections) or degraded implant material (3 sections). Degradation products were detected inside the tendon tissue in only one section. In 52.8% of magnesium and 65.6% of titanium sections, the tendon tissue was found to be in close contact with the surrounding bone, and in some parts, it was directly inserted into the bone tissue (Mg: 6 sections, Ti: 9 sections). In 6 sections of the magnesium group and 12 of the titanium group, lamellar or woven bone, with bone-forming activity, was observed in the tendon tissue.
3.6. The extent of osseous integration of the magnesium screw increased from week 4 to week 24

The extent of contact between bone and implant increased in the magnesium group from 4 to 24 weeks, especially in the lateral cortical bone. In contrast, the percentage of bone contacting the implant remained more or less stable, except for the medial cortical bone at 24 weeks after implantation (Fig. 11). At 4 weeks, the extent of contact between bone and implant in the intermediate and medial locations was significantly lower in the magnesium group than in the titanium group.

3.7. Fluorochrome staining revealed bone remodeling

The titanium implants were observed to be mostly surrounded by the bone tissue, which indicates an early bone growth directly at the surface of the implant (Fig. 12). The magnesium samples showed more extensive growth of bone tissue at greater distances from the implants than did the titanium groups. In the area directly connected to the implants, the bone tissue was stained with xylenol orange.

4. Discussion

In the present study, a magnesium-based screw was tested and compared to a titanium-based screw for potential clinical applications in the reconstruction of the anterior cruciate ligament. The amount of bone which has grown around the implant is one important aspect that was analyzed. Fluorochrome staining showed bone remodeling and new bone formation close to the implants for both the magnesium-based and titanium-based screws. Newly formed bone around magnesium-based implants was described in other studies by intravital labeling [15,26]. In the current study, the magnesium samples demonstrated bone formation after only 4 weeks of implantation. The percentage of bone tissue which had grown around this implant (i.e., the bone-implant contact) was quite low compared to the titanium group at 4 weeks after implantation. For longer implantation times, the direct bone to implant contact increased in the magnesium group while it remained more or less stable in the titanium group.

Another feature analyzed was the degradation rate of implanted material. The general degradation rate of the implanted magnesium alloy was 0.17 mm a$^{-1}$ based on the values of the 24-week group. The difficult part of determining and comparing degradation rates is the variability in implant location, methods applied to determine the degradation rate, animal models and time periods across the different studies. The in vivo corrosion behavior of magnesium alloys is strongly dependent on the influence of numerous environmental factors such as the location of the implantation as well as the corresponding flow and composition of body fluids [27]. Degradation is known to be faster in areas with high body fluid circulation rates (i.e., the marrow cavity) than within the cortical bone tissue [28,29]. Other in vivo studies reported degradation rates of different magnesium alloys between 0.15 and 1.5 mm a$^{-1}$ [27,30]. These rates are lower than the reported in vivo average degradation rates of 2.32 mm a$^{-1}$ for Mg–6Zn implanted for 14 weeks in the femoral shaft of rabbits [28] and of 1.7 mm a$^{-1}$ for ZK50 implanted for 3–4 weeks in the femoral mid-diaphyseal region of rats [31]. Note that the degradation rate in the present study was determined volumetrically, whereas measuring the loss of mass of the material is another method that is being used [32]. In the latter method, the implant has to be removed from the implantation site; this procedure might damage the surrounding tissue and affect the subsequent histological evaluation. Based on the method used in the present study, a precise differentiation between the metallic parts of the screw and the
degradation products is rarely possible, as described in the literature [33]. Therefore, the actual degradation rate of this magnesium alloy might be higher than the estimated rate reported here.

Gas cavities were detected using micro-CT scans with the magnesium alloy samples. These gas cavities appeared mostly around the implants and expanded inside the bone marrow space of the tibia, being most prominent 4 weeks after implantation. The total volume of these cavities decreased significantly from week 4 to week 24. This indicates that the rate at which gas was released decreased with increasing implantation time, and that the gas might be diffused into the bloodstream and extracellular milieu [29]. The cavities were filled up with reproduced bone marrow. Many different studies have also reported the formation of gas during the corrosion of magnesium alloys and the decreased accumulation of the gas after several weeks of implantation [28,34,35]. Thormann et al. reported no proper osseous integration of magnesium alloy (W4; Mg–4Y) implants in macroscopic evaluations of histological slides but did report complete integration of PLLA and titanium screws after 12 weeks [36]. Although most of the magnesium implants after 4 weeks of implantation were surrounded by gas cavities, there was no evidence of detachment or loosening of the fixed tendon. There was also no evidence of lameness in any of the animals. 24 weeks after implantation, in the present study there was a significantly lower amount of gas around the implants; the screws were integrated in the cortical bone and had formed a stable connection. Extreme bone defects as described in other studies [31,37] caused by fast liberation of gas were not observed in the present study. From a clinical point of view, the formation of gas—especially within the first weeks post-surgery—may at first glance be considered problematic for implant stability and hence for the overall procedure since the initial strength of the implanted fixation is crucial for a successful outcome of an ACL reconstruction surgery. Nevertheless, 24 weeks after implantation, all Mg screws were well integrated, and neither signs of tendon loosening nor of necrosis could be found. Moreover, the initial formation of gas in our study did not seem to have a negative effect on the autograft tendon. While there is currently little evidence that formation of gas affects the stability of the tendon, such an effect needs to be further investigated, by additional biomechanical in vitro and in vivo tests under corrosion conditions to determine whether magnesium implants should be considered for clinical use.

The magnesium alloy MgYREZr used here was also previously investigated in different animal studies [16,17] and no histopathological changes in organs or changes of blood values were found. The degradation rate of the magnesium alloy seems to be dependent on its
physiological environment [38]. Different implant locations may result in different release rates, therefore, blood samples were taken to monitor the release of alloy elements, especially yttrium and zirconium, into the circulation. As reported in different studies, particles of the corrosion products could enter the bloodstream and end up accumulating in target organs such as the liver, kidneys and lymph nodes [39–42]. A study by Haley showed degeneration processes of the liver caused by rare earth elements (REE) [43]. Zirconium, in particular Zr4+, at concentrations of over 1000 μM, reduces the in vitro viability of bone-related and vascular-related cells, indicative of its mild toxicity [29]. Obando-Pereda et al. investigated the reaction of zirconia (ZrO2) and titanium particles in mouse calvaria. The zirconia particle-induced pro-inflammatory gene expression was lower, and less osteolysis was found, than in the titanium group. In vitro titanium and zirconia particles significantly increased the expression of cytokines such as TNF-α, IL-1β and IL-6 in cultured macrophages [44]. In the present study, the results of the blood samples showed an increase of zirconium levels 4 weeks after implantation, with a decline after 12 weeks to normal values. There were no pathological disorders in any of the organs in both groups. Based on these results, organ pathologies caused by increased zirconium levels are not expected in blood. These results correspond with the results of other authors [16,17,45–47].

5. Conclusions

The present pilot study in rabbits investigated the magnesium alloy MgYREZr with respect to its potential applicability as implant for reconstruction of the anterior cruciate ligament. Clinically no rupture or loosening of the tendon was observed. The histological evaluation of the magnesium screws showed neither inflammatory reactions nor necrosis of the tendon, and showed a sufficient connection with the surrounding bone tissue 24 weeks after implantation. The results were similar to those of the titanium control. The amount of gas in the implant’s proximity decreased significantly within 24 weeks after implantation. Further biomechanical in vitro and in vivo tests under corrosion conditions are planned to investigate the stability of the bone-tendon construct. The techniques used may need to be modified to further reduce the amount of gas liberated.

Competing interests

Dr. Arne Lucas is employed by the company Syntellix AG, Hannover; he neither influenced the collection of data nor their interpretation. The other authors have no competing interests.

Acknowledgments

We thank Mattias Reebmann, MAike Haupt and Diana Strauch (Hannover Medical School) for excellent technical support. The authors acknowledge the financial support given by the German Research Foundation (DFG) within the collaborative research project SFB 599. We gratefully acknowledge Christopher Müller for the design of Fig. 2 and Michael Schwarze (both from Hannover Medical School) for the statistical analysis. Furthermore, we gratefully acknowledge Dr. Dirk Schaudien from Fraunhofer Institute Hannover for the helpful discussions regarding historical interpretation.

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