Magnesium-Based Intramedullary Nailing System in a Sheep Model: Biomechanic Evaluation and First In Vivo Results

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Rec date: July 17, 2014 Acc date: Sep 19, 2014 Pub date: Sep 21, 2014

Abstract

The tibia is one of the most frequently fractured bones in humans as in animals, and is predominantly treated by intramedullary nailing. However, existing intramedullary nailing systems are consisting of non-degradable material leading to a second surgery for removal. Therefore degradable implant materials as magnesium alloys have been part of the scientific research for years. Especially the slow degrading magnesium alloy LAE442 showed a good biocompatibility but has not been examined as an osteosynthesis system yet. The present study assessed the in vivo applicability of an intramedullary interlocking LAE442 nail system (nail Ø 9 mm; screw Ø 3.5 mm). Four-point-bending tests and finite element simulation were performed. The possible influence of fixation screw holes orientation on the experimental results was tested (change of Young’s modulus during degradation). In vivo biomechanical reliability of the nailing system was examined in a pilot study (sheep, 24 weeks) with clinical, radiographic and computed tomographic investigations. After euthanasia additional micro-computed tomographic, histological and biomechanical examinations were carried out.

Four-point-bending tests (three nail-screw compounds) exhibited a stiffness of 2179.34 N/mm (MV). Bending simulations showed none influence of the fixation screw holes orientation but a decreasing bending stiffness over time.

Simulations of bone-nail assembly indicated highest stress levels within the two distal fixation screws. Clinically the implanted nailing system was tolerated well. Radiographs showed a moderate occurrence of gas and periosteal ossification. Computed tomography hardly detected any decrease of nail and screws volume and density. Comparing four-point-bending after explantation to non-implanted nails (MV) revealed a clear reduction in stiffness to 1769.03 N/mm (18.83%). Histological examinations indicated no significant inflammatory reactions, but predominantly a clear bone-implant interface and fibrous tissue around the central nail. LAE442-based intramedullary nailing system might be an alternative to existing implant materials.

Keywords: Intramedullary nailing; In Vivo; Magnesium alloy; Biocompatibility; LAE442; Sheep; Tibia; Ovine; Bone

Abbreviations
cm: Centimeter; CT: Computer Tomography; °C: Degrees in Celsius; e.g.: For Example; g/mol: Grams per Molecule; GPA: Gigapascal; Hz: Hertz; HU: Hounds Field units; H2: Hydrogen; H2O: Water; i.m.: Intra Muscular; i.v.: Intra venous; kg: kilogram; GY: kiloGray; KV: Kilovolts; kVp: Kilo Voltage peak; l/min: Liters Per Minute; LAE442: Lithium four weight percent, Aluminum four weight percent, Rare earths two weight percent; mAs: Milli Ampere Seconds; Mg: Magnesium; Mg(OH)2: Magnesium Hydroxide; mg/kg: Milligram per Kilogram; mm: Millimeter; mm/s: Millimeter per Second; mm3: Cubic Millimeter; MPa: Megapascal; ms: Milliseconds; MV: Mean Value; N: Newton; n: Number; N/mm: Newton per Millimeter; RIA: Reamer Irrigator Aspirator System; SD: Standard Deviation; SV: Score Value; vol%: Volume Percent; wt%: Weight Percent; YA: Micro Ampere

Introduction

One of the most commonly used methods for treatment of closed and open fractures of the (lower) extremities is the use of intramedullary nailing (internally fracture restoration) [1-4]. For both, humans as well as animals, the tibia is the main bone of the lower leg and can be referred to as one of the most frequently fractured long bone in addition to the radius. Furthermore, tibia fractures in humans are the most common indication necessitating fixation of fracture for early post surgical load bearing [5-7]. Therefore, the optimal fracture restoration of the tibia is of prime importance. The surgeon has to choose between a variety of geometries and different types of intramedullary (interlocking) nailing systems, which were selected according to specific criteria. The most specific criteria are bone length and diameter of the bone (additionally, inter alia physical condition, fracture type and location have to be taken into account) [7]. However, conventionally used implant materials have disadvantages. They can cause stress shielding effects [8-11] and intolerances [10,12,13], which lead to implant revision in a second surgery, connected with the well-known risks and damages for patient and bone. Furthermore, the related significant amount of costs for the health care system should also not been ignored [14,15].
For rendering the second surgery unnecessary, there had been an intensive investigation of biodegradable materials. Magnesium alloys as resorbable implant materials have been part of scientific research for several years [16-18] however, no intramedullary osteosynthesis system has been investigated in vivo yet. Magnesium is an essential element of the human body and earlier studies could exclude allergic reactions [19,20]. Moreover, magnesium showed osteoconductive capability during previous investigation [17,21-24]. Due to its low initial stiffness and furthermore the reduction of stiffness over time, the effect of stress shielding (degradation of bone due to the missing load bearing) could be reduced or even avoided [25]. Several different alloys have already been examined [26-28]. Predominantly rare earth-containing or calcium/zinc-containing alloys are recommended as biocompatible [19,23,29-31]. Compared to other alloys containing rare earth elements, the alloy LAE442 showed the slowest degrading properties and the lowest influences on surrounding tissue [32,33] as well as promising behavior in rabbits [34-38].

To assess the feasibility of a new implant system prior to an in vivo study, biomechanical testing as well as mechanical simulations in terms of finite element analysis are suitable [39,40]. Four-point-bending is a well-established method to determine the interesting specimen properties (bone as well as implants) [41]. Compared to tensile tests, the advantage of the four-point-bending is that it does not require special end geometry of the specimens and the fact that it is frequently used for square and round rods. Due to its larger area of a constant bending moment (between the two force transmission points), and with that reducing the deviation of the maximum bending moment, four-point-bending is superior to three-point-bending [42]. To complement the pure material parameters obtained by four-point-bending, mechanical simulations (finite element analysis) nowadays are a versatile calculation method and an important tool for theoretical review of physical processes [43]. Due to the possibility of a mathematical calculation, mechanical performance of structures can be predicted without the expenses of manufacturing and in vivo testing a part. That leads to a considerable reduction of product development time [44]. Therefore, it could be tested first, if the samples provided for the study are sufficiently stable enough, and if different bending positions of fixation screw holes’ influence the four-point-bending results.

For a following pilot study, the sheep model was chosen which a commonly used model is in the orthopedic research for several years. The sheep with its tibia size and its weight is almost comparable to long bone in humans. Additionally due to similarities with humans in bone and joint structure as well as bone remodelling process, the sheep is useful to address biomechanical, biochemical and histological processes of bone biology [45]. Furthermore, a very congruent reaction to the seated damage in sheep allows an easier interpretation of the experimental results [46-48].

Aim of this study was to examine the newly developed LAE442-based intramedullary nailing system biomechanically and as well in a first in vivo pilot study in a sheep model to establish the experimental design for a comprehensive animal study.

Material and Methods

Implant material

In this study LAE442 intramedullary nails and screws were examined. The alloy material LAE442 was fabricated by the Institute of Material Science (Leibniz Universität Hannover, An der Universität 2, 30823 Garbsen, Germany). After the casting procedure as described in a former study [49], an extrusion process (320°C, stamping speed 2 mm/s, extrusion from 120 mm to 10 mm) was performed to improve mechanical and corrosion characteristics [34]. The exact alloy composition adjacent to magnesium was verified using inductively coupled plasma spectroscopy (lithium 3.7 wt%; aluminum 3.62 wt%; rare earths: neodymium 0.16 wt%, cerium 0.73 wt%, lanthanum 0.38 wt%, praseodymium 0.03 wt%). Thereafter nails and screws (nails: diameter 9 mm, length 130 mm, four locking holes, two at the proximal and two at the distal end, each pair arranged 90° shifted to each with a diameter of 4 mm; interlocking screws: shank diameter 3.8 mm, thread diameter 3.5 mm, core diameter 2.9 mm, length 15 – 40 mm, thread pitch 1.25 mm) were manufactured by the Institute of Production Engineering and Machine Tools (Leibniz Universität Hannover, An der Universität 2, 30823 Garbsen, Germany) by using the CTX 420 linear lathe (Gildemeister, Bielefeld, Germany). After the process of production, the implants were cleaned by acetone for analysis (58.08 g/mol, Merck KGaA, 64271 Darmstadt, Germany) for 45 minutes (including 15 minutes of ultrasonic bath), thoroughly rinsed in deionized water, dried at room temperature and individually packaged before sterilization with gamma radiation (> 25 kGy; BBF-Sterilisations service GmbH, Kernen, Germany). A total number of four nails were used in this study (three for biomechanical testing before implantation, one for 24 weeks implantation in sheep tibia and following biomechanical testing after explantation).

Biomechanical examination of the magnesium based intramedullary nail

All mechanical tests were performed by a material-testing machine (MTS 858 Mini Bionix, MTS Systems, Eden Prairie, USA 55344; controller: Flex Test 40; program: multipurpose test ware, MTS Systems GmbH, Hohentwielsteig 3, 14163 Berlin) following the Standard Specification and Test Methods for Intramedullary Fixation Devices (ASTM F1264-03 (reapproved 2007)) [41]. Using this hydraulically operating testing system, material reactions to axial load (n=3) were examined. The MTS device consisted of two parts: The upper part transmitted the force onto the testing jig (Figure 1). The lower part served as support for the specimen. A displacement transducer was positioned below the centre of the nail to record the actual deflection. The specimen was lying on round support (manufactured by the Hannover Medical School research laboratory, Carl-Neuberg-Straße 1, 30625 Hannover) in horizontal direction (Figure 1). An anterior-posterior force direction was selected. The test sequence was performed until fracture without a preload. The load was applied displacement controlled (0.01 mm/s) and the testing data was recorded at 100 Hz. Stiffness, Young’s modulus and the maximum breaking strength were calculated by using the method of the least square error.

Finite element simulation methodology of the loaded intramedullary nail

Numerical simulations of the four-point-bending tests were performed using ABAQUS’ version 6.11-3 (Dassault Systemes, 78140 Velizy-Villacoublay, France) to examine the influence of fixation screw holes orientation on the results of the experiments. Therefore, models with different orientations of the intramedullary nail were generated. The support and loading rollers were simulated as rigid bodies (Figure 2). Frictionless contact was assumed between each roller and
the nail. In the middle of the nail, the translation normal to the bending direction was prohibited for a small area in the centre. The support rollers were fixed in each direction. As the experiments showed, the yield point is about 1000 N. To ensure that the simulated deformation is within the elastic range, the loading rollers were loaded with a force of 450 N each. A Young’s modulus of 43.5 GPa and a Poisson ratio of 0.35 [50] were assumed for LAE442.

Figure 1: Four-point-bending of an intramedullary magnesium-based nail with the 858 Mini Bionix material-testing machine. A: Nail before testing: inner drill holes positioned exactly on the support rollers in horizontal direction, the two outer drill holes are not visible. B: Nail after testing: typical u-form and breaking point, which occurred after each testing.

The model with a parallel orientation of the fixation hole to the bearing roles was chosen to figure out how the Young’s modulus must have changed over the cross section for different assumptions of the degrading behavior, which takes place during an in vivo implantation.

It was assumed, if there are no significant changes in the diameter of the nail after implantation, but changes in the bending stiffness that corrosion products replace the origin material at the nail surface. Thus, the Young’s modulus of the elements was changed as a gradient from the unmodified Young’s modulus in the middle to the lower adapted to the results of the four-point-bending tests in an iterative process.

In a next step an ovine tibia was scanned with micro-computed tomography (XtremeCT, Scanco Medical, Zurich, Switzerland; voxel size: 82 µm) and a model of a sheep tibia with intramedullary nail was developed on the basis of these scans, using Mimics V14.0 from Materialise (MATERIALISE GmbH, Friedrichshafener Straße 3, 82205 Gilching) (Figure 3). As a control, a four-point-bending test of the bone without a fracture and an intramedullary nail was simulated. The bone was fixed into the direction of bending between the proximal fixation screws and just above the ankle and loaded with 250 N at each loading to get a bending moment in the middle of the bone of 6.75 Nm, which is similar to a study of Gautier et al. [51].

Figure 3: Model of an ovine tibia with the intramedullary nail. Model generation on the basis of micro-computed tomographic scans.

Using the numerical results for the material properties of the intramedullary nail before and after 24 weeks of implantation, the assembly of the nail and the bone was simulated at different stages of the healing process under the assumption, that the Young’s modulus of the nail decreases linear over the healing period. The material parameters for the bone [52] and the material properties of the callus at special stages of the healing process [53,54] were taken from the literature. The composite was loaded in the same way as the control.

**Intramedullary implantation of nail-screw-system in vivo (pilot study)**

The intramedullary nailing system was implanted into the right medullary cavity of the tibia of an adult female black-headed mutton pilot sheep (six years old, 90 kg body weight) with a postoperative follow up of 24 weeks. The animal experiment was authorized according to the German Animal Welfare Act (reference number 33.12-42502-04-11/0327). First, sedation with xylazin (0.10 mg/kg i.m., Xylazin 2%, CP-Pharma GmbH, Burgdorf, Germany) was initiated. General anesthesia was induced by an intravenous injection of propofol (4 mg/kg, Narcofol®, CP-Pharma GmbH, Burgdorf, Germany). After endotracheal intubation anesthesia was maintained by isoflurane (2-4 vol% in oxygen mixture, Isofor®-Janssen, Janssen-Cilag Pharma, Vienna, Austria) directly before drilling. The surgical procedure was performed under sterile conditions. A medial access to the knee joint was created through a 10 cm skin incision and a subsequent transaction of parts of the final chords of the musculus quadriceps femoris (musculus vastus medialis) and musculus gracilis. Afterwards the patella was displaced laterally and the joint flexed at its maximum, which uncovered the area intercondylaris for drilling. The access to the medullary cavity was achieved by using a hollow drill bit (10 mm in diameter). The medullary cavity was reamed to 10.0 mm in diameter by a flexible drill and a guide wire (in 0.5 mm steps, beginning with 8.5 mm). The insertion of the magnesium-based nail was performed with assistance of a special target device (Figure 4). Screws were inserted from...
proximal to distal. Therefore, predrilling (diameter 3.2 mm) as well as monocortical extension (diameter 4.0 mm) was performed and a thread was cut in the opposite cortical bone (diameter 3.5 mm, thread pitch 1.25 mm). For the fixation of the second and the third interlocking screws, temporary displacement of the tibial muscles was necessary. Afterwards, the muscles and the patella were repositioned and the knee joint as well as the wound was closed layer by layer. Antibiotic treatment with Amoxicillin (15 mg/kg/ every other day, Duphamox® LA, Pfizer Animal Health GmbH, Germany) and analgetic treatment with Metamizol-Natrium (40 mg/kg/daily, Metamizol®, WDT, Germany) was administered for 10 days postoperatively.

Clinical examination

The clinical examination included the general condition (posture, behavior, vital parameters: respiratory frequency, heart rate, auscultation of lungs, body temperature; mucous membranes, conjunctivae, rumen auscultation, blood count) as well as a special examination of the wound (reddening, swelling, augmentation, purulence, suture dehiscence, temperature) and the hind limb (lameness, joint filling, comparative palpation of musculus biceps femoris). The first two weeks it was carried out daily, the next six weeks every other day, and from week eight onwards weekly. For semiquantitative analysis of the clinical investigations, the examined parameters were assessed as followed: no occurrence (0), minimal (1), minimal-moderate (2), moderate (3), moderate-high (4), high (5). The evaluation of lameness was examined according to Stashak [55] and Baumgartner [56]. The score was determined as follows: full weight bearing by walking and standing up (0), full weight bearing by walking and high weight bearing by standing up (1), high weight bearing by walking and moderate weight bearing by standing up (2), moderate weight bearing by walking and minimal weight bearing by standing up (3), minimal weight bearing by walking and no weight bearing by standing up (4), no weight bearing by walking and standing up (5).

Radiographic and computed tomographic examination of the tibia:

During the investigation period radiographs have been performed regularly in two planes (without sedation), to record the bone changes on the one hand and degradation related gas formation on the other hand (directly postoperative, first 12 weeks biweekly, then monthly, 35° and 145°, 86 kV, 3.2 mAs; portable x-ray unit “orange 1060HF”, EcoRay Co., Ltd., Seoul, Korea). For radiographic evaluation of bone changes and degradation corresponding gas formation, a semiquantitative scoring system was established (Table 1), which includes the four different sections of the tibia (Figure 4).

Additional computer tomography images were performed in general anesthesia as described above (directly postoperative, then every four weeks until week 16, additionally week 24) to evaluate bone reaction, implant degradation and gas formation. Therefore, the sheep was slid in supine position into a Brilliance 64-layer-CT-system (Phillips, scan protocol: carpus-tarsus, slice thickness: 0.90 mm, 120 kV, 35 mA). Volume (mm3) and density (HU) of the nail, screw one and screw four were evaluated by using an adapted yet another dicom viewer (YaDiV) software program (Institute of man-machine communication, specialized section for graphical data processing, Leibniz Universität Hannover, Welfengarten 1, 30167 Hannover). By analyzing screw one and four, the subsequent comparison to the histological evaluation was reached.

<table>
<thead>
<tr>
<th>Score value</th>
<th>Part a</th>
<th>b</th>
<th>d</th>
<th>Part a</th>
<th>c</th>
<th>Part a</th>
<th>Part b</th>
<th>d</th>
<th>Part c</th>
<th>Part a to d</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>1</td>
<td>0 - 15% of total area, 0 - 6 x 1 - 5 mm</td>
<td>0 - 15 x 0 - 5 mm one side of bone</td>
<td>0 - 22 x 0 - 5 mm one side of bone</td>
<td>0 - 12 x 0 - 5 mm one side of bone</td>
<td>0 - 5 x 0.5 mm</td>
<td>0 - 5 x 0.5 mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Post mortem examination

At the end of the follow up period of 24 weeks, the sheep was euthanized. The anesthesia was induced by an intramuscular injection of xylazine (as mentioned above) followed by an intravenous injection of ketamine/xylazin mixture (7.5 mg/kg Ketamin 10%, 1.12 mg/kg Xylazin 2%, CP-Pharma Partner GmbH, Burgdorf, Germany). Pentobarbital (70.4 mg/kg Narcoren® Merck, Darmstadt, Germany) was injected for euthanasia. Organ samples (liver, kidneys, spleen, cortex cerebri, cerebellum, brain stem and M. tibialis cranialis) were obtained and the right hind leg was explanted.

Examination of the residual nail

The middle part of the nail (as shown in Figure 2) was retrieved from the tibia and stored in silica gel until four-point-bending with the material-testing machine was performed the next day as described above. Stiffness, Young’s modulus and the maximum breaking strength were evaluated by using the method of the least square error.

Partial micro-computed tomographic examination of nail-screw compounds

The areas of screw one and three as well as the area centrically between them were exemplarily scanned in a micro-computed tomography (XtremeCT, Scanco Medical, Zurich, Switzerland; approximately 2 cm each section, voxel size 41 µm, integration time 100 ms, projections/180°: 1000, 60 kVp, 900 µA) for a more detailed visualization of bone and implant structure.

Histology

The bone-implant-compounds of screw one and four were fixed in 4% formaldehyde solution (formalin) at room temperature for seven days. Afterwards they were dehydrated with an ascending alcohol series and embedded in Technovit 7200 VLC (2-Hydroxyethylmethacrylate, Heraeus Kulzer GmbH, Wehrheim, Germany; protocol according to Heraeus Kulzer GmbH Division Technique). Cross-sections of the bone-implant compound were manufactured according to Donath [57] with a slice thickness of 50-100 µm (Figure 4). The histological evaluation was performed descriptively due to the observed tissue reactions after Toluidine blue staining [58] (Toluidine blue O 0.1% Merck, Darmstadt, Germany), with a particular focus on the presence of leukocytes (especially occurrence of monocyte-origin cells, lympho- and plasmocytes) and fibrous tissue as well as new bone formation and remodelling. The slides were evaluated microscopically (Zeiss AxioImager Z1, Carl Zeiss, Jena, Germany) using AxioVision software release 4.8.2 (Carl Zeiss AG, Jena, Germany) using AxioVision software release 4.8.2 (Carl Zeiss AG, Jena, Germany). As magnesium-based implant degradation is possible at any point of fixating and embedding processes. [59] numerous gas bubbles occurred on the slides which were differentiated between in-vivo and ex-vivo origin by forming of fibrous tissue capsules around the in-vivo bubbles.

Table 1: Scoring system for the radiographic evaluation of the digital radiography. Gas inside and outside (area of screw heads) the medullary cavity, periosteal ossification and endosteal clarification were evaluated each in the four different parts of the bone-implant compound.

| Area | 15 - 30% | > 30 x < 20 - 25 mm | > 20 - 25 x > 4 - 6 mm | > 25 x > 6 - 8 mm | > 44 - 65 x > 15 - 20 mm | > 44 - 65 x > 20 - 25 mm | > 24 - 35 x > 10 mm both sides of bone | > 24 - 35 x > 15 - 20 mm one side of bone | > 24 - 35 x > 10 mm one side of bone | > 15 - 30 x > 10 mm both sides of bone | > 15 - 30 x > 5 - 10 mm both sides of bone | > 12 - 24 x > 5 - 10 mm both sides of bone | > 12 - 24 x > 5 - 10 mm one side of bone | > 10 - 15 x > 1 - 2 mm | > 15 - 20 x > 2 - 4 mm | > 20 - 25 x > 4 - 6 mm | > 25 x > 6 - 8 mm |
|------|----------|---------------------|-----------------------|-----------------------|--------------------------|--------------------------|-------------------------------|--------------------------------|-------------------------------|--------------------------------|--------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| 2    |          |                     |                       |                       |                          |                          |                               |                               |                               |                          |                          |                               |                               |                               |                               |                               |                               |                               |
| 3    |          |                     |                       |                       |                          |                          |                               |                               |                               |                          |                          |                               |                               |                               |                               |                               |                               |                               |
| 4    |          |                     |                       |                       |                          |                          |                               |                               |                               |                          |                          |                               |                               |                               |                               |                               |                               |                               |
| 5    |          |                     |                       |                       |                          |                          |                               |                               |                               |                          |                          |                               |                               |                               |                               |                               |                               |                               |
| 6    |          |                     |                       |                       |                          |                          |                               |                               |                               |                          |                          |                               |                               |                               |                               |                               |                               |                               |

Part c: Only little space between bone and nail, evaluation of gas was not possible.

Biomechanical examination

In the four-point-bending, the magnesium-based nails showed a mean stiffness of 2179.34 N/mm (SD 24.69) and a mean maximum force of 3443.61 N (SD 57.08) prior to implantation. For the explanted nail after 24 weeks implantation time 18.83% reduction in stiffness (1769.03 N/mm) and 23.87% reduction of maximum force (2621.68 N) were determined.

Simulation

The simulations showed that the influence of the fixation holes orientation relative to the support rollers is negligible. The material properties within the simulation were adapted to reach a similar relationship before implantation and after implantation as in the experiments. The simulated four-point-bending test of the degraded nails showed that for the assumption of a linear gradient for the Young's modulus a reduction from 43.5 GPa to 33.5 GPa at the surface...
leads to a decrease of the bending stiffness of 17.6%. In the case of a quadratic gradient for the Young’s modulus a reduction to 32.5 GPa leads to a similar bending stiffness. The four-point-bending simulations of the bone-nail assembly showed that the highest stresses occurred at the distal end of the nail and within the fixation screws. The bending stiffness of the bone and the nail was only 11% of the control when the Young’s modulus of the callus area was assumed to be only 1 MPa. After four weeks the bending stiffness increased to 66%. Another four weeks later the bending stiffness was 102%.

### In vivo investigation of the nail-screw system

#### Clinical and radiographic examinations

Clinically, the sheep showed a minimal to moderate (SV 2) swelling and warmth in the wound area within the first two weeks after implant surgery. Within the first week postoperatively, the post surgical lameness reduced from SV 4 to a low-grade lameness of SV 2. Minimal to moderate palpable gas formation (SV 2) occurred between day 14 and 36 at the medial side of the tibia around screw head one and four, followed by minimal (SV 1) palpable gas until day 65. A completely lame-free gait (SV 0) was reached during the 10th week postoperatively, although a partially changed gait remained. Slight muscle atrophy (SV 2) of the musculus biceps femoris was ascertained.

Postoperatively, although a partially changed gait remained. Slight muscle atrophy (SV 2) of the musculus biceps femoris was ascertained. A week after implant surgery, the bone showed clinical and radiographic signs of bone clarification only slight to moderate increasing score values occurring in part b, c and d. At the upper end of the tibia above the unsealed drill hole, a radiopaque cloud-like structure occurred. It was visible from week eight onwards and increased continuously until week 24 up to a size of approximately 0.7 x 0.4 cm.

#### Computed tomographic examinations

##### In vivo computed tomographic examination

The evaluation of the regularly performed computed tomographies showed a slight reduction of implant volume of the three implant components (Table 2). Very limited changes in nail and screws density could be observed during the investigation period. The nail as well as screw four showed a very slight increase of the density in contrast to screw one, which had a very limited decrease (Table 3).

<table>
<thead>
<tr>
<th>Week (mm³)</th>
<th>Nail</th>
<th>Screw one</th>
<th>Screw four</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>6874.72</td>
<td>457.25</td>
<td>317.26</td>
</tr>
<tr>
<td>24</td>
<td>6733.72</td>
<td>437.66</td>
<td>297.88</td>
</tr>
<tr>
<td>Decrease (mm³)</td>
<td>140.99</td>
<td>19.60</td>
<td>19.38</td>
</tr>
<tr>
<td>Decrease (%)</td>
<td>2.05</td>
<td>4.29</td>
<td>6.11</td>
</tr>
</tbody>
</table>

Table 2: Implantation time dependent changes in volume. The exemplary parts of the implanted intramedullary nailing system (nail, screw one, screw four) were evaluated by the use of CT-data (Brilliance 64-layer-CT-system, Phillips). A slight decrease in volume was observed for all three selected parts of the implant.

<table>
<thead>
<tr>
<th>Density (HU)</th>
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<tbody>
<tr>
<td>Week</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>24</td>
</tr>
<tr>
<td>Decrease (HU)</td>
</tr>
<tr>
<td>Decrease (%)</td>
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</tbody>
</table>

Table 3: Implantation time dependent changes in density. The exemplary parts of the implanted intramedullary nailing system (nail, screw one, screw four) were evaluated by the use of CT-data (Brilliance 64-layer-CT-system, Phillips). A slight decrease in density could be observed for screw one.

Comparison of screw-nail compound of screw one and four at week zero (directly after implantation) and week 24 by the Phillips CT-system showed newly formed periosteal bone mainly around screw four, whereas screw one was not completely integrated in bone tissue.
Gas formation was predominantly visible inside the medullary cavity and surrounded the screw heads in lower quantities. The in vivo computer tomography results confirmed in the additional micro-computer tomography scans after euthanasia. Newly formed periosteal and endosteal bone as well as a tight bone-implant interface could be seen especially around screw three (Figure 7 C). In contrast, screw one was not completely integrated in bone tissue, although partial bone adhesion was visible at the screw threads (Figure 7 A). Gas could be observed in the medullary cavity around the implant in examined parts, very small amounts around the nail (Figure 7 B) and to a greater extent in the areas of the interlocking screws (Figure 7 A and C).

**Histology**

Different cellular reactions were observed. In general, no signs of inflammatory reaction could be seen in the area of the screws. Around the fourth screw the bone-implant interface consisted of newly formed bone with lamellae on final stages of remodelling (Figure 8 B, D, F and G), whereas a gap appeared between the bone and screw one (Figure 8 A). In the bone marrow cavity, in dependence on the location, various amounts of fibrous tissue could be determined, surrounding the central implant nail (Figure 8 C and H). In the area of screw four, the fibrous tissue occurred as a thicker layer and closer to the endosteal cortex and formed cavities around the central nail (Figure 8 H). Insignificant inflammatory reaction was observed, limited to small areas of fibrous tissue. The cellular infiltrate consisted predominantly of lymphocytes with some adhesion of macrophages. In the area of screw one, the fibrous tissue formed a tiny layer adherent exactly to the surface of the nail and the surface of the screw (Figure 8 C). Bone marrow, presented mainly by fat tissue, was present in the trabecular bone of the metaphysis in the area of screw one, and in small amounts among the fibrous tissue in the bone marrow cavity in the area of screw four, closer to the endostium (Figure 8 B).
Discusssion

The aim of the study was the examination of a potential magnesium-based intramedullary nailing system (LAE442 alloy) concerning biomechanical parameters, a corresponding finite element simulation as well as an exemplary in vivo degradation behavior and biocompatibility evaluation in the sheep.

For an initial appraisal of the applicability, biomechanical testing is a well-established and commonly used method for assessing material properties in biomedical devices [41,61-63]. Furthermore, experimental mechanical data are indispensable to refine material-based finite element simulations, which can be used to predict material behavior under different loading conditions and during the degradation process.

Compared to common-used implant materials like surgical steel or titanium [63,64], the stiffness of the used magnesium based nails is reduced. The maximum force was measured with approximately 3500 N and the deformation of the four-point-bended magnesium nails in the present study already started at a value of more than 1000 N. In contrast, starting plastic deformation of the cylindrical part of a titanium nail (diameter 8 mm, length 420 mm) and its associated bolts is described at a four times higher bending load of about 5600 N [63]. Hsu et al. examined stainless steel nails (nail diameter 3 x 11 mm and 3 x 12 mm, 1.5 mm wall thickness) and retained the ASTM as their reference line too. They received a bending stiffness (6303.71 N/mm MV) three times higher compared to the bending stiffness of the examined material in the present study (2179.34 N/mm MV). Although nail geometry differs in both studies, a less biomechanical stiffness and ultimate strength can be stated for the magnesium-based nails. With this lower stiffness and the additional degradation over time (further stiffness reduction of about 18% during the 24 weeks follow up), undesired stress shielding effects [65,66] might be avoided or at least reduced. However, finite element studies which can detect highest strain areas in implant and bone [67,68] showed, that the initial strength might be borderline for fracture fixation in weight bearing bones. A calculated sufficient stability of the assembly was assumed four weeks after fracture fixation. The implemented four-point-bending simulations of the bone-nail assembly showed an increase of bending stiffness from 11% to 66% within the first four weeks. Several studies tried to determine a method measuring fracture stiffness (healing bone) directly or indirectly on the patient in long bones. They tried to improve the results of surgeries by decreasing the rate of refractures by a too early removal of stabilization [69-72]. Different authors were able to define an endpoint of fracture healing, from which the healing bone should be able to resist full load. This point was determined for bending stiffness by at least 15 Nm/°, amounting to approximately 25% stiffness of the parent bone [69-71]. Högel et al. determined a bending stiffness of about 115% (69 Nm/°) after ten weeks of healing by testing load at yield [73]. This previously mentioned result is comparable to our result, calculated by mechanical simulation, showing 102% after eight weeks. Flörkemeier et al. compared bending stiffness of distracted to physiologic sheep tibiae and determined in the physiologic tibiae a two and a half times higher stiffness for torsion and almost two times higher stiffness for bending at day 74 postoperatively [74]. The averagely regained stiffness in comparison to the physiological bone resulted in 39.7% for torsion and 56.2% for bending. These results were much lower compared to ours (102% after eight weeks, calculated by mechanical simulation). A possible explanation could be the fact that a distraction of bone has been carried out by which the bone healing would be protracted. To bridge the time gap, an additional stabilization (e.g. external cast) or a reduction in weight bearing (by hanging in a suspension device) for the first weeks might be required to reduce implant loading in fracture fixation. The computation generated expected reduction of stiffness of about 17% over time was very close to the actual achieved value of 18.83% during the implantation period and associated degradation in the in vivo situation. Therewith, the used method is particularly suitable for the chosen LAE442 implant even to predict degradation properties.

Compared to other studies with LAE442 a reduction in ultimate strength of 23.87% maximum force after 24 weeks seemed to be low. Intramedullary implanted LAE442 pins in rabbit tibiae showed a reduction in ultimate strength of 47% after six months [75]. However, the implant geometry as well as the animal model was different and direct comparison questionable. As described in the literature, [47,76] the bone healing processes are much faster in rabbit than in sheep, which could lead to a higher material degradation rate. Secondly, degradation progress from the surface [77] and an increased diameter might lead to a decreased material degradation, as the implant material in relation to the bodyweight is approximately more than three times higher in the present sheep study than in previously performed studies in rabbits. Furthermore, different bending tests were performed, which can further influence the results. In general, a slow degradation rate is desired to guarantee a sufficient long time of implant stability, to avoid a high amount of degradation products as well as gas formation, [75,78,79] and to prevent local alkalinization around the implant to ensure pH dependent physiological reaction balances [80]. Although a stiffness reduction about 18% after 24 weeks could be observed, computed tomographically evaluated volume loss of the nail was only about 2%. A possible hypothesis for variable decreases in stiffness, volume and density could be a kind of diffusion of Mg ions through the magnesium oxide layer, which is already described during high temperature corrosion [81]. Another probable explanation might be an unequal degradation of elements with higher and lower density and different material properties, the elimination of individual ions or the replacement of the origin material by endogenous elements with a lower density and different bending properties (e.g. phosphorus) [77]. Until today the in vivo degradation process of the implant material has not been fully understood and further research is necessary in this field.

Considering the feasibility, the operation procedure with the special target device could be considered as relatively fast and standardized realizable, although the second screw was dislocated. This might be due to a widened gliding hole and therefore a deviating advance direction. However, due to the unloaded implant situation and the focused issue on implant degradation and biocompatibility, the dislocated screw was not determined as relevant. The operation procedure has recently been performed in other studies similarly [46]. Reaming, which was performed in the present study, is discussed very controversial in the literature because of the potential damage of the supplying blood vessels. Klein et al. [84] assumed a better perfusion in the cortical bone (endosteal tenth and periosteal third) acutely after a non-reamed nailing compared to RIA reaming. Reichert et al. [85] could not find a significant alteration of the blood flow in the cortical diaphyses after reaming the medullary channel (despite the absence of intramedullary blood source after reaming), but a significantly increased blood flow in the periost. Both indicated to an acute reversal of direction of blood flow in cortical bone in answer to the caused injury. Biomechanically, reaming extends the bone to nail contact area and reduces the annular gap, which decreases healing time due to a
more rigid fixation [40]. Due to the very diverging opinions, a final assessment of the influence of reaming on bone is not feasible, but a possible influence on degradation rate of the implant, due to the reduction of endostal blood circulation could not be excluded and should be kept in mind.

With regard to the biocompatibility of the magnesium-based nailing system, the occurred reddening and swelling of the wound and the surrounding tissue could be considered as usual tissue response due to the surgery procedure [86,87]. Radiological visible bone remodelling processes could be observed in an acceptable range. Periostal ossification as well as endostal clarification occurred mainly at the distal end of the nail and might be caused by different factors, influencing bone remodelling processes. First, in this region, highest nail to bone contact as well as mechanical stress occurred [88]. Secondly, newly formed bone might have been induced by osteoconductive characteristics of the magnesium alloy and emerging degradation products [17,21,24,89]. Third, even the implantation process itself could already have initiated bone reactions [35]. The radiographically visible cloud-like structure above the unsealed drill hole might be the reason for the remained partially changed gait of the sheep. A possible explanation could be a kind of mechanical obstacle, which leads to a slightly shortened inseam and thus to a slight muscle atrophy of the musculus biceps femoris. Newly formed bone around head and tip of screw three, a moderate gas formation around the nail head and tip of screw three, a moderate gas formation around the nail was found in micro-computed tomographic slices. A corresponding result was seen histologically. Screw four was surrounded by newly formed bone, which indicates good osteoconductivity of LAE442 [60]. The inflammatory reaction was insignificant and limited to small areas of fibrous tissue. However, the space around the central nail was partially empty and bordered by fibrous tissue. A tissue, which has to be considered as not desirable, due to its participation in a foreign body reaction and potential negative effects to bone [90-92]. Furthermore a gap was seen between screw one and the adjacent bone. Diverse histological appearance may result from various corrosion rates of magnesium alloy in different parts of the bone [60]. Whereas empty areas may additionally appear on histological slices as a processing artifact of fixation [59] and cutting-and-grinding technique [93,94], they can also be caused by gas cavities which resulted from the implant degradation process. Radiologically visible gas formation occurred from the second week after implantation till the end of the investigation period in different amounts inside and outside (screw heads) the medullary cavity (Figure 5), but without clinical relevance. Gas formation in magnesium based alloys during the in vivo degradation process is a commonly described phenomenon [23,82,95-97] and is caused by the overall corrosion reaction: Magnesium plus two molecules of water react to magnesium hydroxide and hydrogen (Mg + 2H₂O -> Mg(OH)₂ + H₂) [98-100]. Although the occurring gas is expected to diffuse or to be resorbed to a certain extend [23,30], the continuing existence of gas cavities due to ongoing degradation processes might have induced the formation of fibrous tissue layers around the nail and the incomplete bone ingrowth in the area of screw one.

Conclusions

The examined slow degrading LAE442-based intramedullary nailing system might be an alternative implant material to reduce stress shielding effects during fracture healing and to avoid a second surgery. However, initial stiffness might be border line and an additional initial stabilization could be necessary. The clinical compatibility of the intramedullary nailing system was good and osseointegration of the interlocking screws could be found with exception of parts of the first screw. However, some fibrous tissue and mild inflammatory changes of the medullary cavity were observed. For a more detailed assessment of the biocompatibility, further studies are necessary. Though, this pilot study is the basis for following extensive examinations of the developed LAE442-based intramedullary nailing system.

Acknowledgements

This study is part of the collaborative research centre (CRC 599, Medical University of Hannover, University of Veterinary Medicine Hannover and Leibniz University of Hannover), which is founded by the German Research Foundation (DFG) [INST 192/168-3]. Special thanks to Melanie Kielhorn for excellent technical support.

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