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Pre-clinical testing of human size magnesium implants in miniature pigs: Implant degradation and bone fracture healing at multiple implantation sites

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ABSTRACT

Two miniature pig models to assess safety and performance of degradable osteosynthesis implants are presented. Both models provide multiple implantation sites with human size implants. In the first model, different types of magnesium plates and screws for fracture fixation were used to study local and systemic safety aspects in 14 Göttingen minipigs. Implant degradation, gas release and accumulation of alloying elements in organs were assessed for non-coated and plasmaelectrolytic coated magnesium implants and compared to the titanium reference. The observed implant degradation was mostly uniform and did not seem to depend on the implantation site and implant condition. The coating was effective in delaying initial gas release and degradation. No rare earth alloying elements could be detected in local lymph nodes, kidneys, livers or spleens.

In the second model with Göttingen und Yucatan minipigs, full osteotomies were inflicted to four different anatomical sites and treated with magnesium plates and screws to assess fracture healing performance. Two Göttingen pilot minipigs showed promising results including a mandible osteosynthesis which healed within 6 weeks. The subsequent study was compromised by the more massive jaws of the used Yucatan minipigs. Three out of seven animals had to be sacrificed within two months as the stability of magnesium and titanium reference implants in the mandible was surpassed.

In conclusion, the resorbable magnesium implants showed promising in vivo properties. For the analysis of human standard sized implants under full chewing load conditions, lighter Göttingen minipigs were more suitable than heavier Yucatan minipigs.

1. Introduction

Animal studies are important for assessing safety and performance of new implant designs and are typically required by authorities for certification. This is especially true in case of envisioning new degradable implant types. For preclinical studies in orthopedics, an ideal animal model would allow transferability of results to human patients while needing a minimum of animals and resources [1]. In the case of degradable implants [2,3], small animals might be used for assessing safety but would not be adequate to evaluate performance if mechanical loading is involved [4–6].

Transferability might be achieved if the metabolism of the animal is as human-like as possible. Besides the influence of metabolism on degradation rate, the combined effect of degradation and mechanical loading could lead to premature failure due to environmentally assisted cracking [7]. Such effects are particularly important in osteosynthesis [8], where mechanical support of the implants for the stabilization of bone fractures is crucial to achieve performance, i.e. bone healing. When investigating bone fracture healing with an animal model, the rate of bone turnover should be similar to human bone [9] and the metabolism of the surrounding soft tissue should induce comparable degradation of the implants [10]. In addition, the use of human size implants and human-like surgical techniques should be possible. After implantation, those implants should be submitted to a mechanical loading situation which resembles the human environment. For example, if a mandibular fracture should be investigated, ruminants like sheep or goat should not be used as the mechanical load situation is fundamentally different, in particular regarding fatigue resistance. Skeletally mature miniature pigs - however – are not ruminants, but omnivore and have a temporomandibular joint, which is relatively

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similar to the human counterpart. Minipigs with human-like weight and adequate biting forces are better suited to investigate mandibular fractures and other cranio-maxillofacial applications [11,12].

The present paper will provide an overview of two types of studies with miniature pigs, both using magnesium alloy implants at multiple implantation sites and under different load situations:

- A first study with 14 Göttingen miniature pigs aimed at implant degradation to show safety
- A second study with 2 Göttingen and 7 Yucatan miniature pigs aimed at bone fracture healing to show performance

Implantation site specific results of these two studies have partially been published previously [13–17]. In order to provide a more general picture, the present paper focuses on non-published aspects related to the following research questions:

1. Was degradation depending on implantation site and implant condition?
2. Did rare earth elements from the used magnesium alloy accumulate in organs?
3. Is the miniature pig an adequate model to test bone fracture healing with human size implants?

2. Materials and methods

2.1. Magnesium alloy and reference materials

A magnesium alloy based on the composition of WE43 (chemical composition: magnesium-yttrium-neodymium-heavy rare earths) was used. Compared to the compositional ranges for WE43 (according to the standard ASTM B80), the used alloy - which was developed and manufactured by Magnesium Elektron (Swinton, England) – had a lower impurity level. The raw material was delivered as extruded bars with appropriate diameter for subsequent machining (6–12 mm). The used reference materials have been described earlier (titanium [14,16], PLGA [17]).

2.2. Implants

With the exception of rivet screws [14], all reference implants were commercially available products used in human surgery.

All magnesium implants were machined out of extruded bars using conventional techniques (milling, drilling, threading). Machining was carried out dry, i.e. without using liquid lubricants and/or cooling emulsions.

An overview of the implants used for the first minipig study is given in Table 1. Dimensions of the magnesium implants were identical to the titanium reference except for the titanium rivet-screws which were smaller in diameter and wall thickness [14].

Table 2 and Fig. 1 show the implants used for the second minipig study. The dimensions of the magnesium implants were adjusted according to the chosen reference materials. In order to achieve equivalent bending stiffness, magnesium plates were ~1.5 times thicker than titanium plates and ~1.5 times thinner than PLGA plates. Screw diameters were adjusted to allow similar insertion torque than for the reference screws (i.e. ~1.5 times larger than titanium and ~1.5 times smaller than PLGA). Table 3 illustrates the effect of these design principles on the implant dimensions. The magnesium screws with 1.5 mm diameter were equipped with a torque limiting neck and the larger magnesium screws with torque limiting drives which plastically deformed when a given torque was surpassed.

The aim of using material specific dimensions was to achieve comparable fracture fixation and stabilization. If identical dimensions were used, a different load sharing situation of the osteosynthesis would result and lead to a discrimination of one of the materials. Compared to the PLGA reference, the reduced palpability of the magnesium implants would be an advantage. Compared to the dimensions of the titanium reference, the exposed surface of magnesium implants increased by about 30%. As a consequence, the associated gas release was expected to increase accordingly and the total degradation time was expected to be prolonged by a factor 1.5 due to the thickness increase.

2.3. Coating, cleaning and sterilization

A plasmaelectrolytic coating with a lean electrolyte according to PCT-patent WO 2013/070669 was used with direct current, a maximum current voltage of 400 V and current density of 0.014 A/m^2. The resulting coating thickness was about 10 µm. Surface roughness increased due to the coating [15]. The locking screw threads and plate counterparts remained uncoated by applying the coating with the screws fixed to the plates.

After the coating, the implants were sonicated in 98% ethanol, dried in air, vacuum packaged in a double pouch system and γ-sterilized with a dose of 25–29 kGy.

2.4. Animals

The cohorts of the two animal studies are listed in Table 4.

For the first study, 14 skeletally mature Göttingen minipigs aged about 3 years of both sexes and with an average weight of 53 ± 7 kg were used [13–16].

For the second study, only skeletally mature, female minipigs of at least two years of age were used in order to single out gender specific effects and to minimize the study size. Two pilot animals from a Göttingen breed (same supplier as in the first study, weights 46 kg and 56 kg) and seven animals from a Yucatan breed (weights 70–93 kg) were used. The two pilot Göttingen minipigs underwent surgery at the University Hospital in Berne (Switzerland), in accordance with the Swiss animal protection law. Both animals were sacrificed six weeks

Table 1
Location of implantation sites and implant types used for the first minipig study. Dimensions of the magnesium implants used for the safety evaluation. The corresponding titanium reference implants had identical dimensions except for the rivet-screws which had smaller diameters. Plate dimensions are given as length x width x thickness. Screw dimensions are given with the thread diameter Ø and the total length L.

Site	Plate type	Plate dimensions/mm	Screw type	Screw dimensions/mm
Nasal bone	Rectangular	60 × 6 × 1.5	no screws (Fixation with sutures)	
Frontal bone	6 holes, thick	60 × 6 × 1.5	Cortex Non locking	Ø2.0 L6
Frontal bone	8 holes, thin	40 × 5 × 0.9	Cortex Non locking	Ø2.0 L6
Mandible	no plates (standalone screws)		Rivet screw 0.165 mm wall thickness	Ø2.43 L6 Ø2.53 L6
Rib arches	6 holes, thick	47 × 7 × 1.8	Cortex Non locking	Ø2.0 L6

Table 2
Location of implantation sites and implant types used for the second minipig study. Dimensions of the magnesium plates and screws used for the evaluation of fracture healing performance. Plate dimension are given as length x width x thickness. Screw dimensions are given with the thread diameter Ø and the total length L.

Site	Plate type	Plate dimensions/mm	Screw type	Screw dimensions/mm
Supra-orbital rim	2 holes with bar	14.1 × 5.1 × 0.6	Cortex Non locking	Ø1.5 L4 – L6
Zygomatic arch	8 holes with bar	23.1 × 5.1 × 0.8	Cortex Non locking	Ø1.5 L4 – L6
Mandible (cranial)	4 holes with bar thin	29.7 × 5.75 × 1.45	Cortex Locking	Ø2.7 L6 – L20
Mandible (caudal)	4 holes with bar thick	31 × 7.0 x 1.8	Cortex Locking	Ø2.7 L6 – L20
Rib arch	4 holes with bar	50 × 9 × 2.25	Cortex Non locking	Ø3.2 L8 – L12

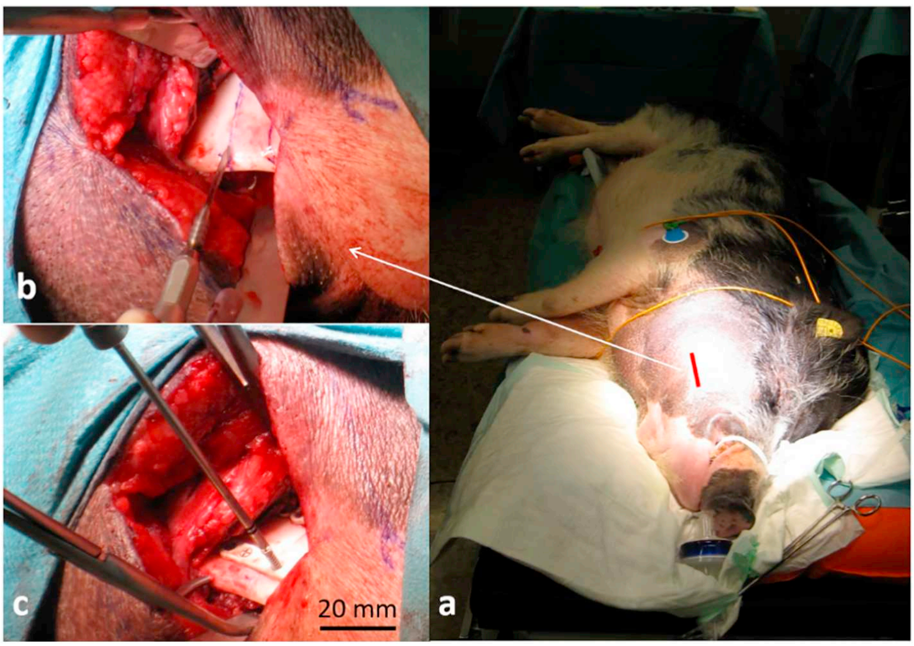

Fig. 1. Surgery of a Göttingen minipig: a) Animal on the operation table with red line marking the position of the first incision. b) Preparation of the mandibular bone for the osteotomy. c) Insertion of a bicortical magnesium locking screw. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Comparison of the screw diameters and plate thicknesses of magnesium and reference implants used for the second minipig study.

Location	Reference plate thickness/mm (material)	Magnesium plate thickness/mm	Reference screw diameter/mm (material)	Magnesium screw diameter/mm
Supra-orbital rim	0.8 (PLGA)	0.6	Ø1.5 (PLGA)	Ø 1.5
Zygomatic arch	1.2 (PLGA)	0.8	Ø2.0 (PLGA)	Ø 1.5
Mandible (cranial)	1.0 (Ti)	1.5	Ø2.0 (Ti)	Ø 2.7
Mandible (caudal)	1.25 (Ti)	1.8	Ø2.0 (Ti)	Ø 2.7
Rib arch	1.5 (Ti)	2.25	Ø2.9 (Ti)	Ø 3.2

Table 4
Animal distributions for the first and second minipig studies. Number of Göttingen (Göt) and Yucatan (Yuc) minipigs used per time point.

Animal model	Time of euthanasia/ months	Non coated magnesium	Coated magnesium	Reference material
First (Safety)	3	3 Göt	3 Göt	1 Göt
	6	3 Göt	3 Göt	1 Göt
Second (Performance)	1.5 (pilot)	–	1 Göt	1 Göt
	1*	–	1 Yuc	1 Yuc
	2*	–	1 Yuc	–
	9	–	2 Yuc	1 Yuc

after surgery.

Due to the availability of skeletally mature minipigs in North America, the study protocol was changed from Göttingen minipigs to Yucatan minipigs. The animal experiments with the seven Yucantan minipigs [17] adhered to protocols approved by the Institutional Animal Care and Use Committee (IACUC) of the preclinical testing facility (AccelLAB, Boisbriand, Quebec, Canada) which is certified by both the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) and the Canadian Council on Animal Care (CCAC). One of the animals with magnesium implants had to be euthanised after nine days due to a displaced mandible and was excluded from the study. Due to badly healing mandibles, three other animals were sacrificed before the initially planned 9 month period. As a consequence, the animal distribution had to be modified to the time points listed in Table 4.

In order to exclude potential interactions between magnesium and

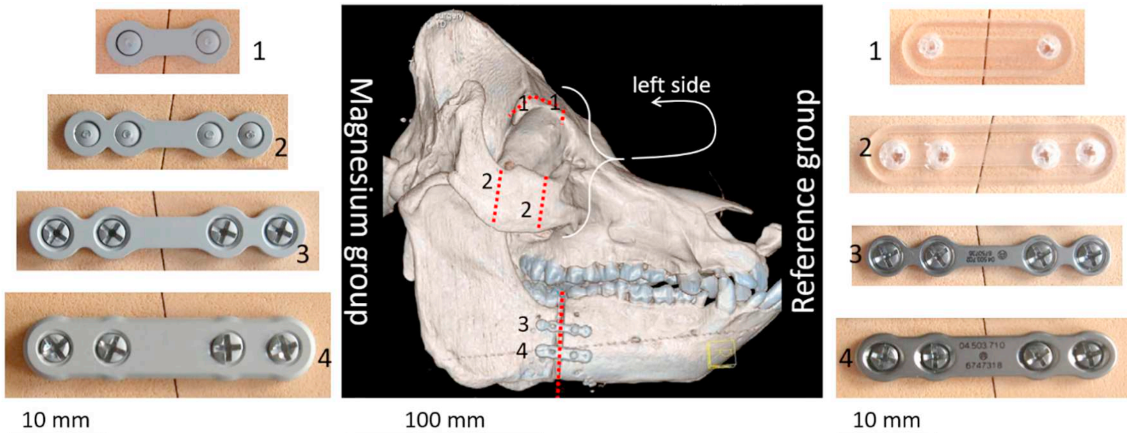

Fig. 2. CT reconstruction of the skull bones of a Yucatan minipig. Overview of implantation sites, osteotomies and used implants for the performance study. The red dotted lines show the osteotomy lines. The numbers indicate the positioning of the corresponding plates: plate 1 (orbit, left side), plate 2 (zygoma, left side), plates 3 & 4 (mandible, right side). The rib specific plate is not shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

reference materials, the magnesium groups only received magnesium implants and the reference groups only reference materials (i.e. titanium and PLGA).

2.5. Surgical procedure

The performance of an osteosynthesis procedure might be evaluated by assessing the bone healing of an osteotomy. Dividing a bone with a cut (i.e. inflicting an osteotomy to the animal) is a common method to simulate a bone fracture. The separated bone pieces are surgically fixed with implants to restore mechanical integrity of the skeletal tissue. If the cut bone surfaces are re-set in close contact, the mechanical load will be distributed among bone and implants (load sharing situation). If the cutting ends of the bone are not in contact, the entire load will be borne by the implants (load bearing situation) [8]. The surgeries for both animal studies were carried out by the same team of two Craniomaxillofacial surgeons under the assistance of veterinaries. The surgical procedure for both animal studies has been reported previously [13–17], except for the mandibular and rib osteotomies of the second study.

The first osteotomy was inflicted to the mandibula as follows (Fig. 2):

Starting at the mandibular angle, a 10 cm long horizontal skin incision was made (Fig. 1a). Thereafter, the soft tissue and masseter muscle were partially dissected at the level of zygoma. No impairment of the chewing function was expected as the remaining muscle and the opposed masseter were still intact. The periosteum of the mandible was cut, and the mandibular bone prepared towards the upper rim using a rasp. A vertical line was marked at 15 mm behind the last tooth of the lower jaw (Fig. 1b). The vertical osteotomy was performed using an oscillating saw and the two mandibular fragments were mobilized. According to the principles used for fracture fixation in humans, the two fragments were fixed with two plates: a thinner plate above and a thicker plate below the midline (plates 3 and 4 in Fig. 2). After bringing the bone fragments with two forceps into contact (repositioning), the upper plate was pre-bent to follow the mandibular anatomy. The two holes closest to the osteotomy line were predrilled and two monocortical screws were inserted. In case of the magnesium screws, an additional pre-tapping was applied. The two outer empty plate holes were then predrilled and two monocortical screws were inserted and locked to the plate. The lower plate was pre-bent and fixed in the same manner using bicortical screws. With the completion of the load sharing osteotomy (Fig. 1c), the forceps could be removed.

After the mandibular osteosynthesis, the animal was flipped over to the other side and four additional osteotomies were made: one at the orbit, two at the zygoma and on at the 7th rib (Fig. 2).

In contrast to the earlier safety study [16], only the 7th rib was treated and received a complete instead of a partial osteotomy. The two pilot Göttingen minipigs were treated with the thicker mandible plate and the seven Yucatan minipigs received the longer, rib specific plate for the fixation of the osteotomy (Table 2).

2.6. Radiological examination

Radiological examination of the first animal study has been reported previously [13–16].

For the second study, radiographs of face and chest were made right after surgery using a mobile C-arm [17]. After euthanasia, a CT scan of the head and rib was performed. The 9-month cohort of the Yucatan minipigs listed in Table 4 underwent additional live CT scans at 1, 2 and 6 months. In vivo CTs at regular intervals were aimed at tracking the progress of bone healing and therefore to minimize the number of animals needed.

2.7. Euthanasia and necropsy

Euthanasia and necropsy of the 14 Göttingen minipigs used for the safety study have been reported previously [13–16]. Four organs (local lymph node of the neck, liver, kidney, spleen) were excised from each animal and stored at −20 °C until chemical analysis with ICP-MS.

For the performance study [17], portions of the left zygomatic and left supraorbital rim bones, mandible and rib were dissected from the surrounding connective soft tissue and individually harvested with the implants. Each bone was labeled and fixed in neutral buffered formalin for 24 h and then transferred to a 70% ethanol solution.

2.8. Micro-CT

The bone blocks containing the implants were scanned using a micro-CT scanner (SkyScan model 1172; Bruker, Kontich, Belgium) at 70 kV using a 140 mA X-ray source and a resulting 17 μm resolution [17]. The radio-opaque, metallic implants were aligned perpendicularly to the axis of the X-ray beam.

2.9. Histologic processing

For the safety study, histological processing was done according to Ref. [15] and using MacNeal's tetrachrome. For the performance study,

Table 5
Overview of the results of the first minipig study grouped by implantation site. Outcome summary of the safety evaluation with publication references.

Implantation condition	Degradation/load situation	Safety aspect	Outcome	References
Nasal bone: Rectangular plate inside a tissue pocket	Degradation without load	Uniformity of degradation	Little localized corrosion	[13]
		Strength retention	80% of initial bending strength after 12 weeks	
		Gas release	Coating avoids initial peak release	
Frontal bone: Contoured plates fixed with non-locking screws	Plates fixed against bone, screws under tension	Rupture of screw head during implantation	Failed screws could be overdrilled and replaced	[15]
		Gas release	Depended on screw location	
		Plate-screw connection	No preferential degradation observed	
Mandible: Thin walled rivet-screws pressing against cortical bone	Plastically deformed implant under load	Effect of plastic deformation and load	Coating was effective in delaying gas released despite the plastic deformation	[14]
Rib arches: Contoured plates fixed with non-locking screws	Plates fixed against bone, screws under tension, moving ribs, one with partial osteotomy	Effect of breathing movement and animal weight	Mg mandible plate used for one pilot animal broken	[16]
			Rib specific plates intact, some broken screws	This publication
Systemic effects: all implants combined	Total amount of degradation products	Accumulation of alloying elements in the organs	No accumulation except for Zr	This publication
		Gas release	No systemic effect observed	

Table 6
Chemical analysis of 56 organs retrieved from the 14 minipigs of the safety study. Contents of magnesium in mg/kg as determined by ICP-MS in liver, kidney, spleen and neck lymph node at 12 and 24 weeks for titanium and magnesium implants.

Material	Titanium		Magnesium non-coated		Magnesium coated	
Organ	12 weeks (n = 1)	24 weeks (n = 1)	12 weeks (n = 3)	24 weeks (n = 3)	12 weeks (n = 3)	24 weeks (n = 3)
Liver	211	163	170	164	154	159
Kidney	158	110	127	121	123	121
Spleen	179	92	151	100	120	109
Lymph node	56	153	149	81	99	79

Table 7
Chemical analysis of 56 organs retrieved from the 14 minipigs of the safety study. Contents of zirconium in mg/kg as determined by ICP-MS in liver, kidney, spleen and neck lymph node at 12 and 24 weeks for titanium and magnesium implants.

Material	Titanium		Magnesium non-coated		Magnesium coated	
Organ	12 weeks (n = 1)	24 weeks (n = 1)	12 weeks (n = 3)	24 weeks (n = 3)	12 weeks (n = 3)	24 weeks (n = 3)
Liver	Below detection limit		0.04	0.07	0.02	0.05
Kidney			0.03	0.03	0.01	0.02
Spleen			0.03	0.01	0.00	0.01
Lymph node			0.02	0.04	0.02	0.03

histologies were prepared as described in Ref. [17] and Goldner's trichrome was used for the staining of the non-decalcified sections.

2.10. ICP-MS of organs

From the 14 animals of the safety study [13–16], 56 tissue samples (4 samples per animal, i.e. one from liver, lymph nodes, spleen and kidney) were analysed by an ISO 17025 accredited laboratory for chemical analytics at Seibersdorf laboratories (Austria). Inductively coupled plasma mass spectrometry (ICP-MS) was used to test the contents of Mg, Y, Nd and Zr of all samples. One sample of each group was also tested for traces of La, Ce, Pr, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu. The tissue samples were therefore defrosted and digested with nitric acid and then filled up with distilled water to 20 mL. The sample weights were between 0.2 g and 1.5 g – depending on the tissue type. The detection limit of the elements listed above was 0.01 mg/kg (ICP-MS equipment: Elan 6100 from Perkin-Elmer).

2.11. Estimation of degradation mass and hydrogen release

For the safety study, the mass loss of the rectangular strength retention plates was determined by HF etching of the corrosion layer [13] and amounted to about 13–14% for both, the coated and non-coated implants after 6 months in vivo. The total degraded mass was estimated by extrapolation of this value to the total mass of all implants which was about 5 g. The total hydrogen release was estimated by assuming that 1 mg of degraded magnesium corresponded to ~1 ml of released hydrogen gas according the electrochemical reaction [18].

2.12. Statistical analysis

For the safety study, only the mean and standard deviations were determined and reported. For the performance study, due to the

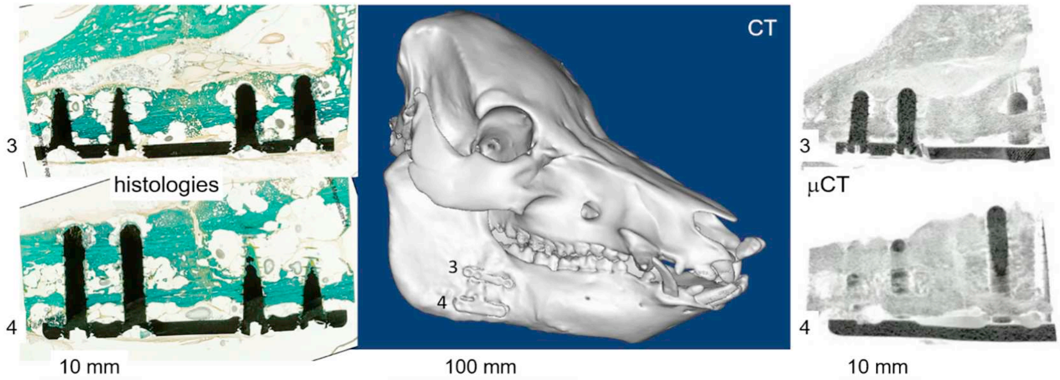

Fig. 3. Göttingen minipig with coated magnesium implants after euthanasia: 3-D CT reconstruction of the skull bones with the magnesium plate 3 and 4 at the mandible osteotomy site (middle). μCT through the screw plane of plate 3 with monocortical screws and of plate 4 with biocortical screws (right). Histologies of both plates with surrounding bone (left).

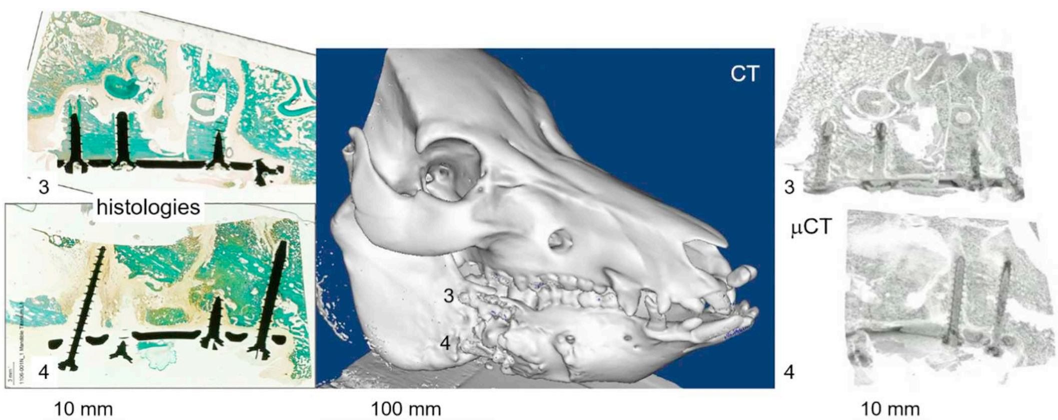

Fig. 4. Göttingen minipig with reference implants after euthanasia: 3-D CT reconstruction of the bone of the skull with the titanium plate 3 and 4 at the mandible osteotomy site (middle). μCT through the screw plane of plate 3 with monocortical screws and of plate 4 with biocortical screws (right). Histologies of both plates with surrounding bone (left).

redistribution of animals, the number of animals per cohort was not large enough to perform valid statistical analyses.

3. Results

3.1. Minipig study for safety evaluation

During the first study with Göttingen miniature pigs, four simultaneous implantation sites with human sized implants were used to assess the safety of magnesium implants with and without coating, whereas titanium served as a reference material. Table 5 gives an overview of the various safety aspects addressed for the different implantation situations and summarizes the outcome. As the results of the individual sites have been published previously [13–17], only unpublished, systemic aspects will be presented and comparisons among sites will be made.

With two rectangular plates on top of the nasal bone, two plates with a total of 10 screws on the frontal bone, four rivet-screws inside the mandible bone and another two plates with ten screws fixed to the ribs, the total mass of magnesium and the large exposed surface was more than a patient typically would get. An estimated total amount of 750 mg (about 15% of the total implant mass) was released within 24 weeks.

It was assumed that degradation products from the magnesium alloy (and from the coating if present) would accumulate inside organs like local lymph nodes, liver, kidney and spleen. The ICP-MS analysis of those organs showed that none of the rare earth alloying elements in scope (Y, Nd, Gd, Dy, Er, Yb) passed the detection limit of 0.01 mg/kg. The measured magnesium levels for animals treated with magnesium and reference implants were in a similar range and reached concentrations between 56 and 211 mg/kg (Table 6). The only other element passing the detection limit was zirconium (Table 7). Even though Zr is not per se an alloying element for WE43, it is commonly used for grain refinement with an addition of less than 1 wt %.

The histological analysis of the organs did not show any pathological changes in comparison with the organs from the two titanium reference animals.

The large exposed surface of all implants presumably led to the release of a relatively high amount of hydrogen gas (about 750 mL within 24 weeks). Besides some limited cavity formation in the tissues surrounding the implants, no systemic effect of the released gas could be observed, neither for non-coated nor for coated implants.

The uniformity of degradation is another safety relevant aspect (Table 5). The in vivo degraded rectangular plates were well intact after 24 weeks of implantation and showed ductile behaviour during flexural testing [13]. The small standard deviation of the bending properties (< 10%) was a sign of negligible localized corrosion, for both, coated and non-coated implants. The occurrence of localised corrosion was sparse also on the other implant types and did not seem to depend on the implantation situation. For the plates fixed to the frontal bone, the plate-screw contact did not lead to preferential degradation. Even the rib plates, which were submitted to presumably larger deformations did not show increased corrosion at the screw heads respectively plate holes. The plastically deformed, thin-walled rivet-screws revealed the

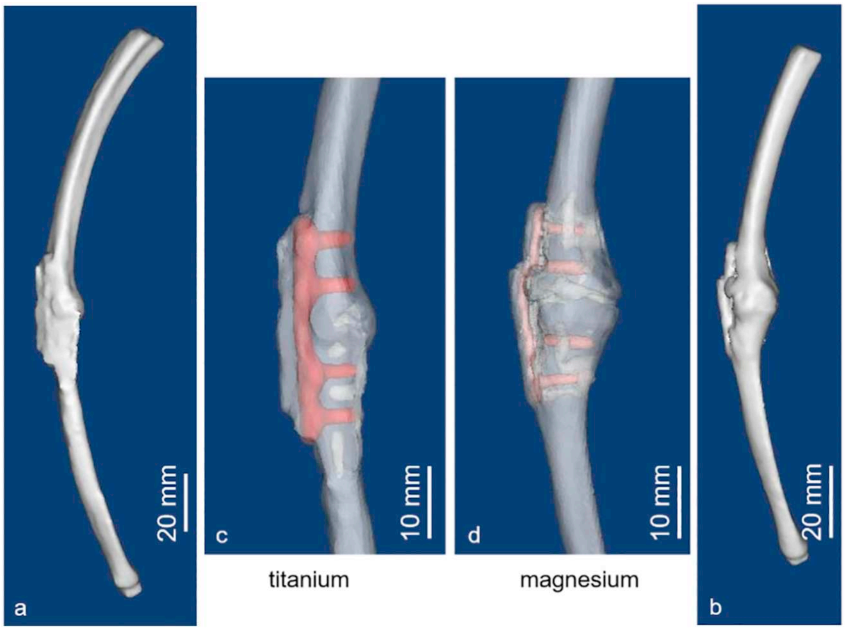

Fig. 5. Osteotomized ribs of the two Göttingen minipigs of the pilot study after 6 weeks (at euthanasia). 3-D CT reconstruction of objects with density equal or higher than bone: ribs plated with titanium (a) and magnesium (b). Semi-transparent view with implants in red with titanium (c) and magnesium (d). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

most pronounced local effects of all tested implants [14], but only beyond their intended time of service. In particular, the non coated rivet-screws were partially degraded after 6 months.

The multisite implantation of human sized implantation covered safety aspects which went beyond the individual implants and allowed insight to eventual systemic effects. Based on these results, the tested alloy could be considered for further evaluation and was rated as save with respect to degradation behaviour and biocompatibility.

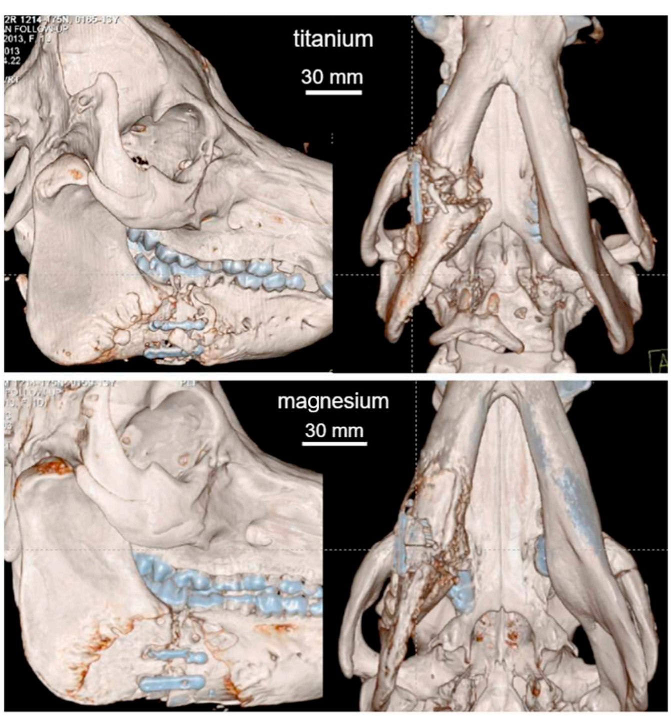

Fig. 6. Yucatan minipigs, re-scheduled to the one-month group, as mandible fixations were becoming unstable after about 4 weeks. 3-D CT reconstruction of the bone of the skull after euthanasia, lateral view (left) and caudal views (right). Dislocated titanium implants, dislocated mandible and comminuted bone (top left and right), thin magnesium plate broken (bottom left) and broken bone piece attached to a magnesium screw (bottom right).

3.2. Minipig study for performance evaluation

3.2.1. Preliminary evaluation with two Göttingen minipigs

The feasibility of this multiple site fracture model was first examined with two Göttingen minipigs as pilot animals. After 6 weeks, the outcome for the supra-orbital rim and for the zygomatic arch was similar for both animals, with an ongoing healing process and disappearing osteotomy lines. Differences between the two animals were seen for the mandible and the rib osteotomy:

The condition of the mandible of the pilot animal treated with magnesium implants was very promising (Fig. 3). After 6 weeks, the osteotomy line was no longer visible. The bone had regained its integrity and appeared to be fully healed. Although degradation of the magnesium implants was not well visible on the histologies, the presence of cavities beneath both plates and around some of the screw tips were a sign of progressing degradation. The occurrence of the cavities, presumably due to gas release, neither seemed to compromise bone healing nor to cause negative side effects. The animal gained about 5 kg

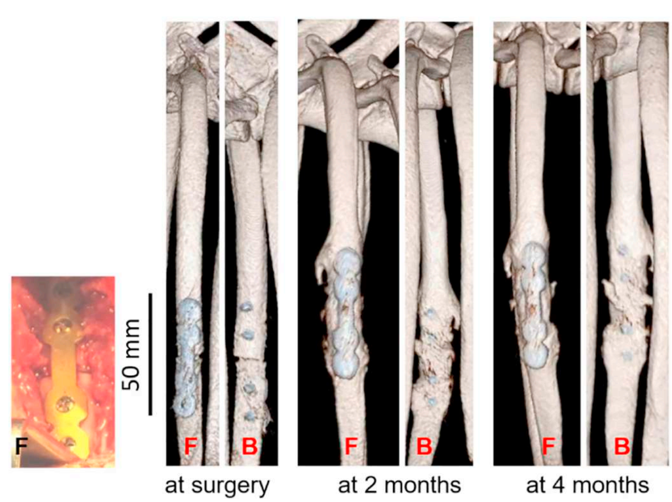

Fig. 7. Osteotomized rib of a Yucatan minipig (performance study) fixed with titanium plate and screws at implantation (left). 3-D reconstruction of objects with density equal or higher than bone with front views (F) and backside views (B). In vivo CTs at 0, 2 and 4 months after implantation.

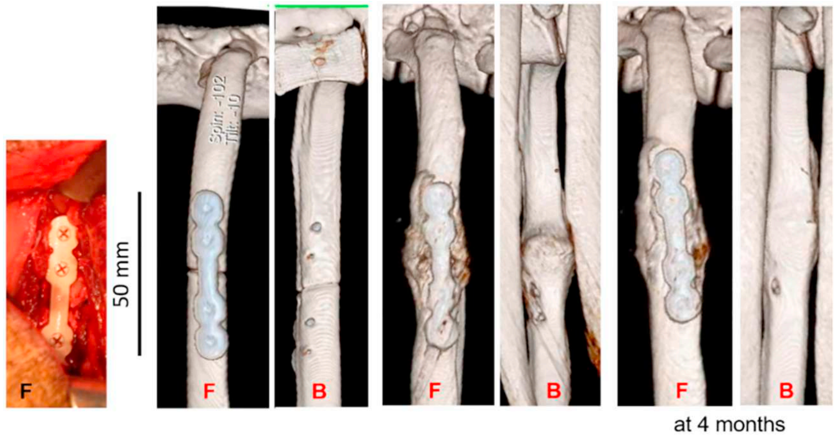

Fig. 8. Osteotomized rib of a Yucatan minipig (performance study) fixed with coated magnesium plate and screws at implantation (left). 3-D reconstruction of objects with density equal or higher than bone with front views (F) and backside views (B). In vivo CTs at 0, 2 and 4 months after implantation.

of weight, from 56 kg at surgery to 61 kg at euthanasia.

The pilot animal treated with titanium implants did not have the expected outcome for the mandible osteotomy. The macroscopic appearance of the bone at the osteotomy was highly irregular (Fig. 4). The integrity of the mandibular bone was compromised as some osteolysis seemed to have occurred. The histological cuts showed that several screws of the bottom plate were disconnected from the plate (Fig. 4, left side). The locking of the screw head with the plate had probably disengaged before healing had sufficiently progressed. Due to the failed mandible healing, the animal lost 3–4 kg of the initial weight by the time of sacrifice.

The osteotomy of the 7th rib was not fixed with a rib specific plate, but with the 1.8 mm thick mandibular plate. For the pilot animal with titanium implants, the CT scan revealed some callus formation at the osteotomy line which was about to disappear (Fig. 5 a, c). The titanium implants seemed intact and partly overgrown by dense tissue, presumably new bone. The callus formation of the rib fixed with the magnesium implants – however – was more pronounced and interpreted as a sign of the instability caused by the rupture of the magnesium plate (Fig. 5 b, d).

From these two pilot animals, it was concluded that the animal model was mechanically challenging, but adequate to test human size implants and that rib specific plates should be used.

3.2.2. Evaluation with Yucatan minipigs

After achieving bone healing in the mandible with magnesium implants, the animal protocol from the preliminary study was extended to a larger number of animals to gain statistical significance of the outcome and to adhere to GLP procedures. Seven Yucatan minipigs underwent surgery, two animals with reference implants and five animals with coated magnesium implants. It turned out that the Yucatan minipigs were larger and heavier than the Göttingen minipigs. The weight of the female Yucatan minipigs at implantation was 70–93 kg [17] instead of 46 respectively 56 kg for the Göttingen minipigs. The increased weight went along with more massive mandibles (Fig. 2), larger masseter muscles and presumably increased biting forces. The large forces involved were evident with the two minipigs whose mandible became unstable after about 4 weeks (Fig. 6). These two animals had to be euthanised earlier than planned and were rescheduled to the one-month group. The animal with the titanium implant had a completely dislocated mandible, with comminuted bone pieces at the osteotomy site and dislocated implants. The animal with

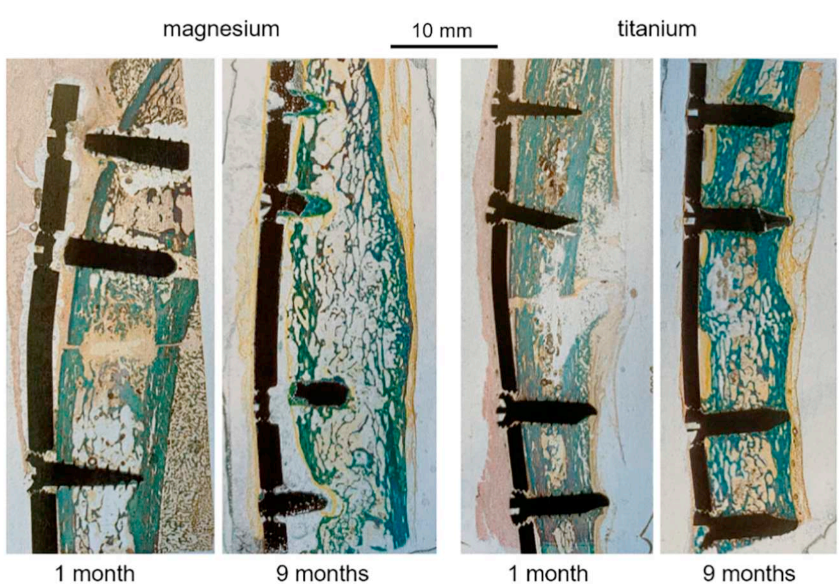

Fig. 9. Histologies of the plated ribs of 1 month and 9 month Yucatan minipigs. Staining with Goldner's trichrome. The osteotomy gap is still visible after one month, with both magnesium and titanium. After nine months, the osteotomy gap is no longer visible beneath either plate.

the magnesium implants had the thinner plate broken and a large, broken bone piece which had been torn off by the magnesium screws of the thicker plate. It was concluded that the involved forces were larger than in human patients where such dramatic failures are not observed.

As a consequence, the model was no longer valid to study a mandibular fracture with human size implants. Despite the imposed rescheduling of the animal groups (Table 4), the six remaining animals could be used to study the outcome of the other osteotomy sites.

The fracture healing and bone remodelling for the two craniomaxillofacial osteotomies have been reported earlier [17].

The healing of the osteotomized 7th rib with rib specific plates was not published yet and is illustrated by CT images of two animals (Figs. 7 and 8). At 4 months, the osteotomy gap is no longer visible for both, titanium and magnesium implants. Instead of a callus formation, the bone around the osteotomy gap of the titanium implant is rather irregular. In the case of the magnesium implant, there is a slight callus formation. The CT results are confirmed by the post mortem histologies shown in Fig. 9. After 1 month, the osteotomy gap is still visible on the Yucatans ribs plated with magnesium and with titanium. After 9 month, the osteotomy gaps are no longer visible in the two animals shown. Unlike the two titanium constructs, screw failures have been observed for magnesium at the level of the screw neck. The locking between screw head and plate did not seem to be compromised for neither titanium nor magnesium.

Although the longer, rib specific plates seemed more adequate than the mandible plates used in the intermediate study, the design of magnesium plates and screws needs further optimization and material specific adaptation in order to avoid failure prior to bone healing.

4. Discussion

Pre-clinical testing of human size magnesium implants in miniature pigs was carried out with two different models, a first model aimed at safety aspects and a second model to evaluate performance of the osteosynthesis. An overview of the performance evaluation outcome is given in Table 8. According to the 3R concept [19], the number of animals could be minimized by using multiple implantation sites. The choice of a large animal was important for simulating human-like in vivo degradation.

depend on the implantation site. This was true for both, coated and non-coated implants. Neither previous plastic deformation (pre-bending of plates, expansion of rivet-screws) nor contact between plate and screw did negatively affect the local degradation. The presumably increased elastic deformation of the rib plates (due to breathing and other movements of the rib cage) did not induce more localized degradation.

The degradation related gas release was most pronounced for the implants with the largest exposed surface ($\sim 18\,cm^2$), i.e. the rectangular plates on top of the nasal bone. The coating was effective in delaying the gas release effectively [13]. Despite a relatively large total amount of released gas inside the organism, no gas related systemic effects were seen. Only local effects like the occurrence of cavities around some of the implants could be observed [14,15].

The second research question - about the accumulation of alloying elements in the organs – was answered by the ICP-MS analysis. Despite a relatively large amount of released alloying elements, no accumulation of rare earth element was found in the organs (lymph node, liver, kidney, spleen). Astonishingly, only the element zirconium, which was used as a grain refiner for the WE43 alloy, passed the detection limit. It can not be excluded, however, that the zirconium originated from other sources. In humans for example, the daily intake of zirconium is 4 mg/day and the amount of zirconium contained in the body about 250 mg [20], which was more than the amount measured in the minipigs.

The third question dealt with the transferability of the fracture fixation animal model to humans. Using a simple cantilever beam model, with forces of 50 N–100 N on the incisors and a plate at 60 mm distance, bending moments of 3–6 Nm were estimated in humans [21]. In the minipigs, the osteotomy gap was about 100 mm away from the incisors (Figs. 2–4). Due to the longer lever arm inside the minipig mandible, the resulting bending moments would be nearly doubled if similar biting forces on the incisors were assumed. As a consequence, the minipig mandible model can be regarded as a worst case test for human size implants. The healed osteotomy of the Göttingen minipig with magnesium implants showed that transferability with humans is possible. However, the choice of the animal breed seemed to have a decisive effect on the outcome of the mandibular osteotomy. Failure occurred with reference and magnesium implants when using the heavier Yucatan minipigs. As the distances between incisors and os-

Table 8
Overview of the results of the second minipig study grouped by implantation condition with description of the degradation and load situation. Performance benchmark of reference implants and outcome summary of magnesium implants with publication references.

Implantation condition	Degradation/load situation	Performance benchmark	Outcome	References
Supra-orbital rim: Full osteotomy (bone piece removed and put back in place)	Small loads Load sharing with non locking plate/screws	Bone healing as for thicker PLGA reference	All osteotomies healed	[17]
Zygomatic arch: Two osteotomies (bone piece stays attached to soft tissue)	Small loads Load sharing with non locking plate/screws	Bone healing as for thicker PLGA reference	All osteotomies healed	[17]
Mandible: Full osteotomy	Chewing forces Load sharing with locking plate/screws	Bone healing as for thinner Ti reference	Göttingen minipig healed with Mg Yucatan minipigs not healed	This publication
7th rib arch: Full osteotomy	Breathing forces Body weight Load sharing and deformations with locking plate/screws	Bone healing as for thinner Ti reference	Some Mg implants failed	This publication

As results of the individual sites were published previously [13–17], the goal of the present publication was to focus on aspects which were systemically relevant and to answer the research questions given in the introduction section.

The first research question was aimed at comparing the degradation of magnesium implants at multiple sites under various implant conditions (load, deformation, screw/plate contact). Both animal studies showed that implant degradation was mostly uniform. The occurrence of localized corrosion was not relevant for the tested alloy and did not

teotomy gap were not fundamentally different between Yucatan (Figs. 2 and 6) and Göttingen minipigs (Figs. 3 and 4), the more massive masseter muscles in the heavier Yucatan minipigs were presumably responsible for the increased biting forces.

In order to corroborate this hypothesis, it would be necessary – however – to measure biting forces for both breeds and to compare them to humans.

Nevertheless, the proposed animal model is attractive for the evaluation of degradable implants for osteosynthesis as several implant systems with different dimensions can be tested in the same animal. Different load situations can be tested with presumably small load in the supraorbital rim and the zygoma. Much higher loads will be encountered at the mandible, where the top plate will predominantly be under tension and the bottom plate under compression. Lateral shear forces due to chewing will also act on the plating system.

When looking at the rib osteotomies, cyclic bending deformations will add to the loading and create a challenging environment for bone healing and implant integrity.

5. Conclusion

The results of the two presented animal models with miniature pigs led to the following conclusions:

1. Degradation of magnesium WE43 implants was mostly uniform and the influence of the implantation site seemed negligible in the chosen model. Neither contact between plate and screw nor plastic deformation during implantation seemed to induce localized corrosion of the tested alloy. The presence of a coating delayed degradation but did not alter the fundamental degradation behavior of the alloy.
2. The degradation of a relatively large amount of magnesium implants did not lead to an accumulation of alloying elements in the minipig's organs (lymph node, liver, kidney, spleen). Only trace amounts of zirconium passed the detection limit of the chemical analysis by ICP-MS.
3. The miniature pig can be a suitable model to study the degradation of human size implants at multiple implantation sites. Safety relevant aspects regarding the release of degradation products can be studied under more realistic metabolic conditions than in a small animal. The miniature pig model for fracture healing was promising to test several implantation sites simultaneously. The heavier Yucatan minipigs - however - were not adequate to study human sized implants in a mandible fracture. The suitability of the lighter Göttingen minipigs for mandible osteotomies needs to be confirmed with additional animals.

Disclosure

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Appendix A. Supplementary data

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References

[1] Y.H. An, R.J. Friedman, Animal Models in Orthopaedic Research, CRC Press LLC, 1999.
[2] F. Witte, Editor's Comment on: the history of biodegradable magnesium implants: a review, Acta Biomater. 23 (S) (2015) S27.
[3] R. Suuronen, R. Kontio, N. Ashammakhi, C. Lindqvist, P. Laine, Bioabsorbable self-reinforced plates and screws in craniomaxillofacial surgery, Bio Med. Mater. Eng. 14 (4) (2004) 517–524.
[4] S.F. Fischerauer, T. Kraus, X. Wu, S. Tangl, E. Sorantin, A.C. Hänzi, J.F. Löffler, P.J. Uggowitzer, A.M. Weinberg, In vivo degradation performance of micro-Arc-oxidized magnesium implants: a micro-CT study in rats, Acta Biomater. 9 (2) (2013) 5411–5420.
[5] F. Amerstorfer, S.F. Fischerauer, L. Fischer, J. Eichler, J. Draxler, A. Zitek, M. Meischel, E. Martinelli, T. Kraus, S. Hann, S.E. Stanzl-Tschegg, P.J. Uggowitzer, J.F. Löffler, A.M. Weinberg, T. Prohaska, Long-term in vivo degradation behavior and near-implant distribution of resorbed elements for magnesium alloys WZ21 and ZX50, Acta Biomater. 42 (2016) 440–450.
[6] A. Chaya, S. Yoshizawa, K. Verdelis, N. Myers, B.J. Costello, D.T. Chou, S. Pal, S. Maiti, P.N. Kumta, C. Sfeir, In vivo study of magnesium plate and screw degradation and bone fracture healing, Acta Biomater. 18 (2015) 262–269.
[7] A. Atrens, N. Winzer, W. Dietzel, P.B. Srinivasan, G.L. Song, Stress Corrosion Cracking (SCC) of Magnesium (Mg) Alloys, Corrosion of Magnesium Alloys, Elsevier Inc., 2011, pp. 299–364.
[8] A. Goharian, Trauma Plating Systems: Biomechanical, Material, Biological, and Clinical Aspects, Elsevier Inc., 2017.
[9] Y. Li, S.K. Chen, L. Li, L. Qin, X.L. Wang, Y.X. Lai, Bone defect animal models for testing efficacy of bone substitute biomaterials, J. Orthop. Transl. 3 (3) (2015) 95–104.
[10] Y. Germanier, S. Tosatti, N. Broggini, M. Textor, D. Buser, Enhanced bone apposition around biofunctionalized sandblasted and acid-etched titanium implant surfaces: a histomorphometric study in miniature pigs, Clin. Oral Implant. Res. 17 (3) (2006) 251–257.
[11] S. Wang, Y. Liu, D. Fang, S. Shi, The miniature pig: a useful large animal model for dental and orofacial research, Oral Dis. 13 (6) (2007) 530–537.
[12] H. Naujokat, J.M. Seitz, Y. Açil, T. Damm, I. Möller, A. Gülses, J. Wiltfang, Osteosynthesis of a cranio-osteoplasty with a biodegradable magnesium plate system in miniature pigs, Acta Biomater. 62 (2017) 434–445.
[13] T. Imwinkelried, S. Beck, T. Iizuka, B. Schaller, Effect of a plasmaelectrolytic coating on the strength retention of in vivo and in vitro degraded magnesium implants, Acta Biomater. 9 (10) (2013) 8643–8649.
[14] B. Schaller, N. Saulacic, S. Beck, T. Imwinkelried, B.T. Goh, K. Nakahara, W. Hofstetter, T. Iizuka, In vivo degradation of a new concept of magnesium-based rivet-screws in the minipig mandibular bone, Mater. Sci. Eng. C 69 (2016) 247–254.
[15] B. Schaller, N. Saulacic, T. Imwinkelried, S. Beck, E.W.Y. Liu, J. Gralla, K. Nakahara, W. Hofstetter, T. Iizuka, In vivo degradation of magnesium plate/screw osteosynthesis implant systems: soft and hard tissue response in a calvarial model in miniature pigs, J. Cranio-Maxillo-Fac. Surg. 44 (3) (2016) 309–317.
[16] B. Schaller, N. Saulacic, S. Beck, T. Imwinkelried, E.W.Y. Liu, K. Nakahara, W. Hofstetter, T. Iizuka, Osteosynthesis of partial rib osteotomy in a miniature pig model using human standard-sized magnesium plate/screw systems: effect of cyclic deformation on implant integrity and bone healing, J. Cranio-Maxillo-Fac. Surg. 45 (6) (2017) 862–871.
[17] B. Schaller, J.P. Matthias Burkhard, M. Chagnon, S. Beck, T. Imwinkelried, M. Assad, Fracture healing and bone remodeling with human standard-sized magnesium versus polylactide–Co-glycolide plate and screw systems using a mini-swine craniomaxillofacial osteotomy fixation model, J. Oral Maxillofac. Surg. 76 (10) (2018) 2138–2150.
[18] G.L. Song, Corrosion Electrochemistry of Magnesium (Mg) and its Alloys, Corrosion of Magnesium Alloys, Elsevier Inc., 2011, pp. 3–65.
[19] W.M.S. Russell, B. R.L, The Principles of Humane Experimental Technique, (1959).
[20] H.A. Schroeder, J.J. Balassa, Abnormal trace metals in man: Zirconium, J. Chronic Dis. 19 (5) (1966) 573–586.
[21] O. Lieger, B. Schaller, A. Bürki, P. Büchler, Biomechanical evaluation of different angle-stable locking plate systems for mandibular surgery, J. Cranio-Maxillo-Fac. Surg. 43 (8) (2015) 1589–1594.