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In vivo degradation of binary magnesium alloys – a long-term study

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Abstract: Bioresorbable magnesium materials are widely investigated because of their promising properties as orthopedic devices. Pure magnesium (99.99%) and two binary magnesium alloys (Mg2Ag and Mg10Gd) were used to investigate the degradation behavior, the bone adherence and bone-implant interface mechanics of these materials in growing Sprague-Dawley[®] rats in a long-term study of 36 weeks. In vivo micro-computed tomography (μ CT) scans were performed at specific time points to observe the longitudinal degradation of each alloy within the same animal. Pin volume and surface, gas volume and degradation rates were calculated. The results showed a slower degradation of pure magnesium and Mg2Ag in comparison to the fast disintegrating Mg10Gd. Changes in bone morphology were determined by high resolution ex vivo μ CT scans and bone sections stained with Toluidine blue. Pure magnesium and Mg2Ag were well integrated and surrounded by bony tissue 24 weeks after implantation. On the contrary, Mg10Gd remnants were surrounded by fibrous and bone tissue.

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Push-out tests revealed higher bone-implant-interface strengths of pure magnesium pins compared to Mg2Ag and Mg10Gd. Mg10Gd induces less beneficial tissue reactions, while Mg2Ag showed adequate biodegradation and no adverse reactions in bone healing process which might be promising as an orthopedic device.

Keywords: μ CT; animal model; gadolinium; magnesium implants; silver.

Introduction

Due to the promising properties of bioresorbable magnesium (Mg) alloys regarding degradation performance, biocompatibility, osteoinductivity and mechanical stability close to bone, several Mg alloys are currently under investigation to be used as medical devices in orthopedic and trauma surgery [1–4]. As a result of a complete degradation of the material, second surgeries for implant removal would be unnecessary [3]. To address uncontrolled high degradation susceptibility, different strategies are currently being pursued. Material purification, processing refinements, surface modifications and alloying strategies with various elements are some options which influence implant degradation velocity [2, 4, 5].

In order to enhance the mechanical and corrosion properties, several studies used Al, Zn, Mn, Ca, Zr and rare earth elements (REE) like Y as alloying elements [6–9]. There are studies using ternary/multiple alloying systems [10–13]. In other cases, Mg binary alloys are used in order to reveal the influence of only one element on the degradation behavior [8, 14] and consequently, these results can be used as guidance for future selection of alloying elements to produce suitable Mg implants [8].

In this study, we chose the binary alloy systems magnesium-silver (Mg-Ag) and magnesium-gadolinium (Mg-Gd) to minimize the influence of multiple alloying elements on implant performance. Ag as an alloying element improves mechanical properties and enhances degradation resistances of Mg-Ag materials after suitable heat treatment. More specifically, the Mg2Ag alloy appeared biocompatible in terms of cytotoxicity and

cytocompatibility in in vitro cell tests and exhibited antibacterial properties in in vitro experiments [15].

The REE Gd is known to lead to increased mechanical properties and corrosion resistance of Mg implants [16–18]. More specifically, a study investigated different concentrations of Gd in Mg alloys (Mg5Gd, Mg10Gd and Mg15Gd) showed that a weight percentage of up to 10% Gd improves the corrosion performance, whereas higher Gd contents (more than 10%) do not enhance the degradation resistance further [16].

In a previous study, the degradation performances of Mg2Ag and Mg10Gd alloys in in vitro and in vivo conditions were compared to pure Mg (99.99%) [19]. Within this 3 months' short-term in vivo study, Mg2Ag showed a slow and homogeneous degradation while Mg10Gd disintegrated completely. However, the influence of the degrading materials on the bone morphology and the long-term bone reactions has not been investigated yet.

This long-term follow-up study aims to investigate the influence of Ag and Gd on the degradation behavior as well as bone adherence and bone-implant interface mechanics in male growing Sprague-Dawley® rats over a period of 36 weeks. The study hypothesis was that Ag and Gd elements can affect the degradation performance of these materials in in vivo conditions.

Our specific research questions are: (i) Is there a significant influence of the alloying elements Ag and Gd on the degradation performance? (ii) How fast do the binary alloys degrade and how long do they maintain their integrity, respectively? (iii) Are there alterations because of the degradation and is the bone able to revert to restitutio ad

integrum after complete absorption of the implant? (iv) Is the micro-computed tomography (μ CT) an appropriate method for evaluating the related tissue response of such temporary implants?

Results

In this study, the sham group was used to observe the normal healing process without implant. In the treated groups, Mg alloy pins of pure Mg, Mg2Ag and Mg10Gd were implanted in order to compare the degradation performance within the bone. The following results elucidate the degradation behavior and the reaction of surrounding bone within the experimental groups pure Mg, Mg2Ag and Mg10Gd in comparison to the drill-only sham group.

Micro-computed tomography

μ CT scans were performed at 1, 4, 12, 24 and 36 weeks to calculate pin volume and surface in order to evaluate the degradation rates of the implants.

The drill hole in the sham group was visible 1 week after the implantation. The bone healed totally upon the 4th week post-operative (Figure 1, Sham).

The μ CT scan images of pure Mg implanted animals (Figure 1) showed close contact between the bone and implant surface 12 weeks' post-operative and consequently new bone formation around the pin surface was clearly obvious 12, 24 and 36 weeks after the operation.

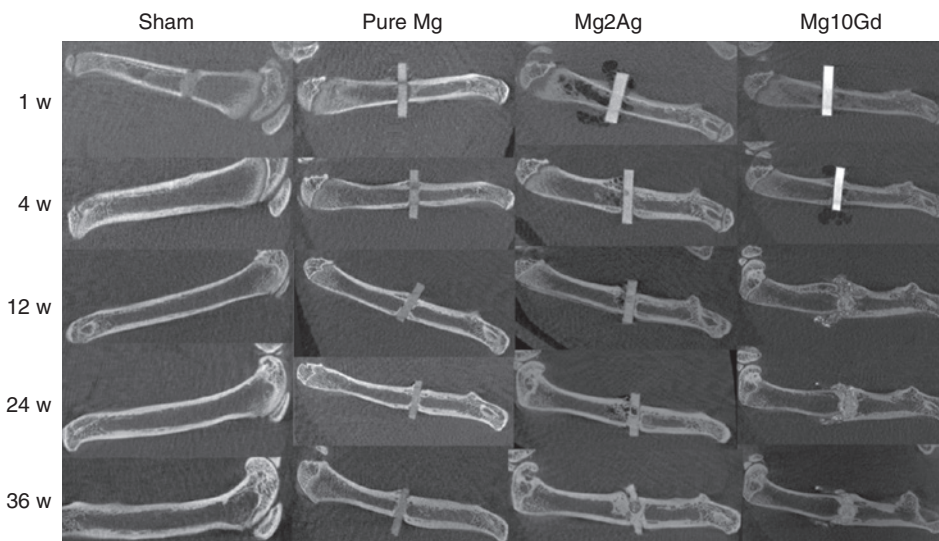


Figure 1: Sagittal μ CT scans showing sham group, pure Mg, Mg2Ag and Mg10Gd at 1, 4, 12, 24 and 36 weeks after implantation in the femoral bone of growing Sprague-Dawley® rats ($n=6$ animals per group).

Within the 12th week after implantation of Mg2Ag, a successful bone and implant surface contact could be detected. This contact triggers the bone adherence and new bone formation around the implant surface which is visible at 24 and 36 weeks (Figure 1).

First bone contact to the surface of the Mg10Gd implants could be seen after the 1st week of the operation. However, after 12 weeks the pins were disintegrated, but new bone formation around the implant remnants was visible 12, 24 and 36 weeks post-operatively (Figure 1).

The volume of the pure Mg pins decreased gradually during the 36 weeks of the study (Figure 2A), but the pin surface remained stable (Figure 2B). Moderate pin volume loss and stable pin surface levels during the entire study indicated that pure Mg exhibits homogeneous and slow-degrading properties. The amount of hydrogen gas evolution was the lowest in pure Mg pins during the whole study period in comparison to Mg2Ag and Mg10Gd and was reduced even further after 4, 12, 24 and 36 weeks (Figure 2C). These results were in accordance with the μ CT images (Figure 1).

The loss of the Mg2Ag pin volume was moderate and linear during the 36 weeks (Figure 2A). The pin surface increased till the 4th week after the implantation but remained at the same levels at the subsequent time points (Figure 2B). Mg2Ag show the highest hydrogen gas formation at the 1st week in comparison to the other materials and was reduced 12, 24 and 36 weeks post-operatively (Figure 2C). At 4 weeks the gas production was at the same level for the Mg2Ag and pure Mg but 12, 24 and 36 weeks the gas formation was significantly higher than the gas formation of the pure Mg. These results were in consistency with the μ CT images (Figure 1).

The pin volume, surface as well as the gas volume of Mg10Gd implants could be only determined for the 1st and 4th week after the implantation and they were not feasible to be calculated after 12, 24 and 36 weeks of implantation. Four weeks post operation the pin volume was decreased (Figure 2A) whereas the pin surface remained at the same level (Figure 2B). At 4 weeks, the gas volume was increased in comparison to the 1st week after the implantation. After 12, 24 and 36 weeks no obvious gas cavities were visible which was consistent with the μ CT results (Figure 1).

Calculation of the degradation rates

The degradation rate of pure Mg started with 0.38 ± 0.12 mm/year at 1st week and decreased to 0.13 ± 0.06 mm/year at 4 weeks after the operation. Further on, it remained

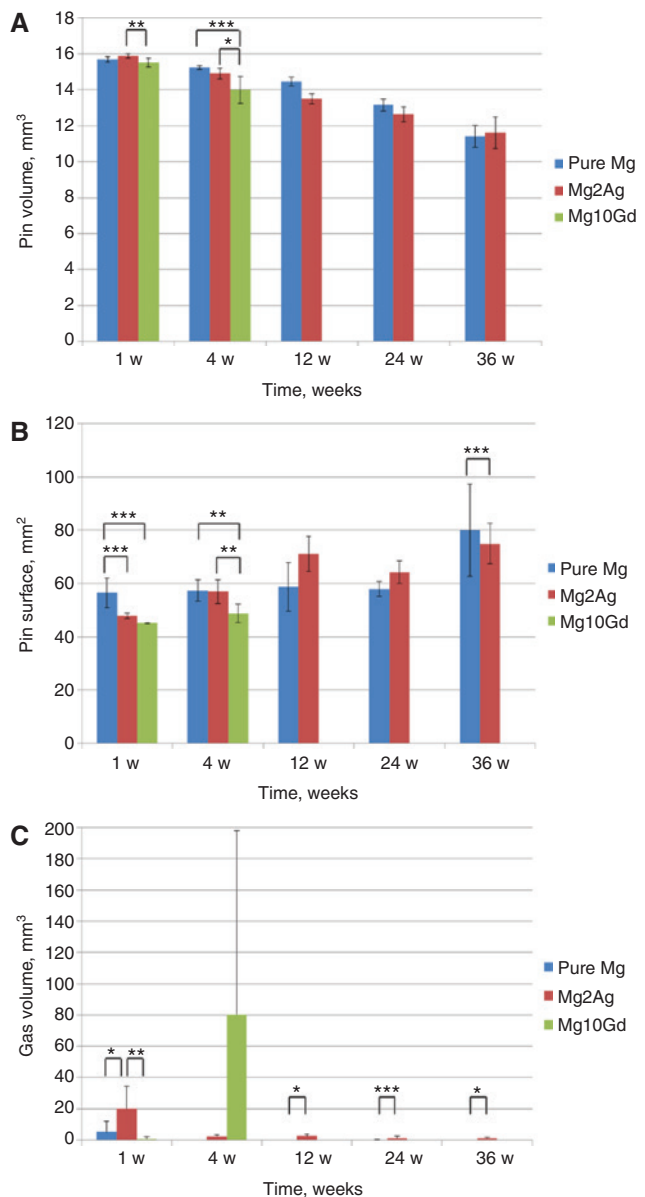


Figure 2: Evaluation of (A) pin volume, (B) pin surface and (C) gas volume of pure Mg pins, Mg2Ag and Mg10Gd at specific time points after implantation ($n=6$ bones/alloy group). The results are expressed as the average \pm standard deviation of the pin volume (mm^3), pin surface (mm^2) and gas volume (mm^3) at time points (weeks). The statistical analysis was performed with the SPSS ANOVA one way Test: *, ** and *** indicate the significant difference of value $p < 0.05$, 0.01 and 0.001 , respectively.

stable until 36 weeks after the operation (0.1 ± 0.04 mm/year) (see Figure 3). This resulted in a slow and moderate degradation performance of pure Mg implants.

The degradation rate of Mg2Ag pins remained stable until 12 weeks post-operative and gradually decreased 24 and 36 weeks after the implantation. Mg2Ag degradation performance was slow and moderate during 36 weeks

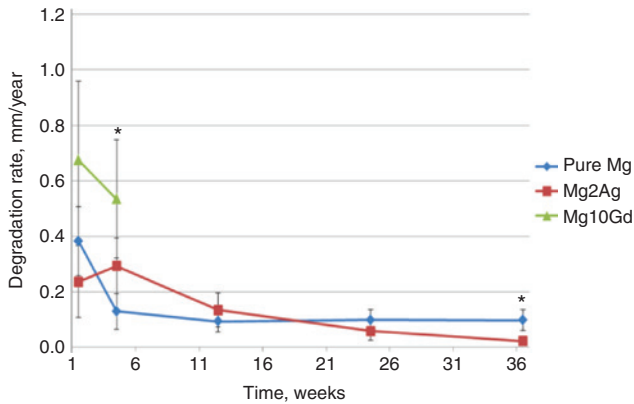


Figure 3: Calculated degradation rate of pure Mg, Mg2Ag and Mg10Gd in the long term in vivo study at different time points ($n=6$ bones/alloy group). The degradation rate of Mg10Gd could not be calculated after 4 weeks due to the inhomogeneous degradation performance and integrity loss of the implant. The results are expressed as the average \pm standard deviation in (mm/year) determined at different time points (weeks). The statistical analysis was performed with SPSS employing post hoc Mann Whitney U test. *Indicates the significant difference in terms of $p < 0.05$.

(initial value: 0.24 ± 0.13 mm/year and final value: 0.02 ± 0.017 mm/year) (see Figure 3).

On the contrary, the degradation velocity of Mg10Gd was 0.67 ± 0.28 mm/year at the 1st week and reduced to 0.53 ± 0.21 mm/year 4 weeks after the implantation. The degradation rate of Mg10Gd was the highest compared to the degradation rates of pure Mg and Mg2Ag at 4 weeks after the operation. Mg10Gd pins lost their integrity after 12 weeks, the pin volume and pin surface was not feasible to be calculated for the subsequent time points. Degradation rates till 12 weeks have already been published by Myrissa et al. [19].

Bone implant interaction

Additionally to the medium resolution μ CT scans, the general bone morphology was investigated by high resolution μ CT scans and histological bone sections were embedded in Technovit 9100. To detect new bone formation around the implant as well as to analyze the implant integration in the bone tissue, the high resolution scans of the embedded samples were compared to the corresponding histological sections.

The μ CT images showed the degradation performance of pure Mg (Figure 4A and C), Mg2Ag (Figure 4E and G) and Mg10Gd (Figure 4I and K) alloys 4 and 24 weeks after implantation. After scanning the tissue, the same blocks were cut and stained with Toluidine blue-O to examine the general bone morphology.

In the pure Mg implanted bone, no gas formation was visible 4 weeks post-operatively. First corrosion pits on the implant surface were visible in the high resolution scans (Figure 4A, orange arrow). Implants were surrounded by fibrous tissue and new formed bone (Figure 4A and B). Twenty-four weeks after the operation, small gas cavities were detectable in the medullary cavity. Pins were surrounded by a thin layer of bone. The green arrows indicate a gap formed between the implant surface and bone visible in Figure 4C.

The Mg2Ag implanted pins did not show any gas formation 4 weeks post-operatively. Signs of first corrosion pits were visible on the implant surfaces that adjoin the intramedullary cavity (Figure 4E, orange arrows). Implants were surrounded by fibrous tissue and new formed bone (Figure 4F). Twenty-four weeks after the operation, small gas cavities were obvious in the medullary cavity (Figure 4H). Again, a gap between the implant surface and bone was obvious (Figure 4G, green arrows).

The Mg10Gd implant showed a small amount of gas in the intramedullary cavity around the pin at 4 weeks after the implantation. Moreover, first degradation of the implant was detectable, especially where the implant was surrounded by tissue (Figure 4I, orange arrow). Twenty-four weeks post-operatively (Figure 4K and L), parts of the disintegrated implant were seen in cranial and caudal sites of the cortical bone as well as within the intramedullary cavity. In addition, these implant particles were encapsulated by fibrous and especially by bony tissue.

Push-out tests

Push-out tests were performed in order to investigate the mechanical strength of the bone-material interface. Figure 5 depicts the energy needed to displace the implanted pin for 0.5 mm in the axial direction. The results of 4 weeks' time points showed that pure Mg pins needed the highest amounts of energy in order to be moved compared to Mg2Ag and Mg10Gd pins referring to a more stable bone-implant interface.

Discussion

As a homogenous degradation performance with a controlled gas release is still a major concern and demanded for the clinical application of Mg implants, several strategies are approached to decelerate the degradation rate [4, 5]. One strategy is the use of alloying elements to

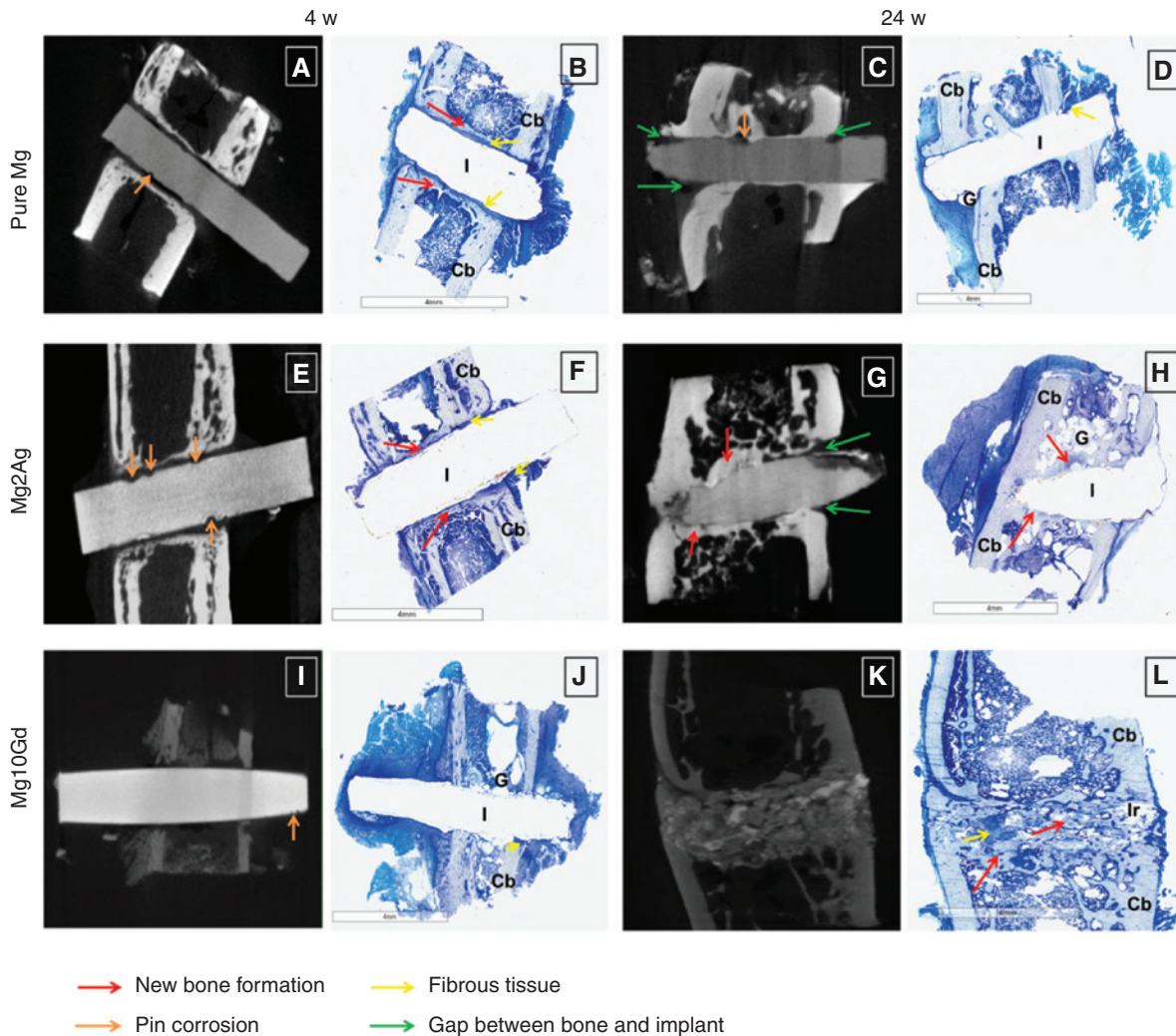


Figure 4: μ CT reconstructed (two-dimensional slices) images of explanted Sprague-Dawley® rat femurs treated with pure Mg, Mg2Ag and Mg10Gd alloys performed with a high resolution protocol at 4 and 24 weeks after implantation. Toluidine blue-O staining was performed using the same samples, showing the general bone morphology during the degradation process. G, Gas bubbles; I, implant; Cb, cortical bone; Ir, implant remnants; orange arrows, pin corrosion; red arrows, new bone; yellow arrows, fibrous tissue; green arrows, gap between bone and implant.

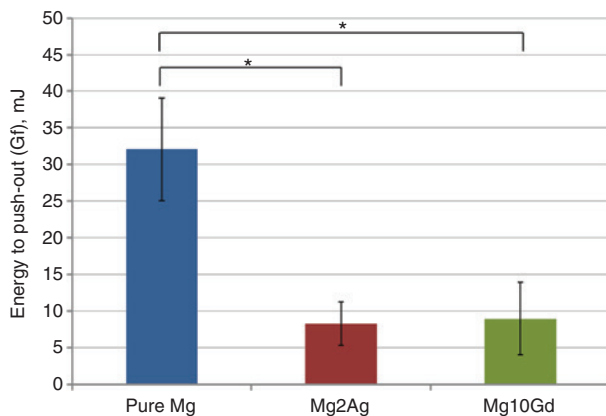


Figure 5: Push out tests of pure Mg, Mg2Ag and Mg10Gd (after 4 weeks) explanted femoral bones (n=6 bones per alloy).

produce Mg alloy that can influence the degradation rate and as a result can guarantee the fracture stability for the required time of bone healing.

In our study, we used binary alloys Mg2Ag and Mg10Gd to avoid the influence of a multiple alloy system. Mg2Ag has been already tested in in vitro cell experiments [15, 19, 20] in which good cell viability was shown [15, 19, 20] and also anti-microbial/anti-bacterial properties were postulated [15]. Regarding the Gd, it is already mentioned that it increases the mechanical properties as well as the corrosion resistance of the Mg alloy [16]. Mg2Ag and Mg10Gd were selected in our study to investigate the performance of the alloy in the bone tissue concerning degradation, gas formation and bone-implant interface up to 36 weeks.

Influence of binary alloys on degradation performance

It seems that Ag and Gd within a binary Mg alloy system affects the degradation rate and has also an influence on the degradation performance of the alloys within an animal model. Pure Mg, Mg2Ag and Mg10Gd showed differences in the degradation performance in the long-term study of 36 weeks. Pure Mg and Mg2Ag revealed gradual pin volume reduction during the 36 weeks (Figure 2A), indicating a homogeneous and slow degradation performance as it is also shown in the literature for different grades of purity of pure Mg [12, 19, 21].

The reduction of impurities to very low values decreases the degradation rate and leads to a slower and homogenous degradation especially in the first phase after implantation [5, 12]. Hofstetter et al. produced and analyzed extreme-high-pure Mg samples with <10 ppm impurity levels resulting in a degradation rate of about 0.010 ± 0.003 mm/year in vitro and 0.013 ± 0.003 mm/year in vivo [12]. Compared to that the used pure Mg in Tie et al. [21] showed initial corrosion rates around 0.9 mm/year and reduced to 0.6 mm/year after 16 weeks of implantation time [21]. Our shown degradation rate of 0.4 mm/year at the beginning and 0.2 mm/year after 36 weeks of implantation and impurities up to 0.0089 wt% are within the range of these published degradation rates and impurity levels.

To date, there is our previous study in the literature investigating Mg-Ag and Mg-Gd alloys in in vivo experiments [19] and the conference contribution of Galli et al. [22]. Within this study, screws made of Mg2Ag and Mg10Gd were implanted in the tibia of rats. The degradation rates 1 month after implantation (Mg2Ag=0.46 mm/year; Mg10Gd=0.78 mm/year) are in accordance to our values of pins implanted in the femur of rats [22]. Furthermore, one recent study regarding the degradation performance of Mg2Ag nail implants was found in the literature [23]. Mg2Ag nails were implanted into the intramedullary cavity of non-fractured femoral bone and fractured femoral bone mice models. They showed an almost complete degradation of Mg2Ag nails by 210 days (30 weeks) and 133 days (19 weeks), respectively, without adverse reaction of bone tissue [23]. Literature about assessing the corrosion rates in vitro of pure Mg, Mg2Ag and Mg10Gd in a 3 days' immersion test is available focussing on the response of human umbilical cord perivascular cells to the Mg-based alloys [20]. Additionally, in vitro corrosion rates of these materials have been studied in a 5 days' immersion test focusing on sterile and unsterile DMEM [24] and in a 10 days' immersion test using DMEM+10% FBS [25].

On the contrary to pure Mg and Mg2Ag, the Mg10Gd pins disintegrated in small particles after 4 and before 12 weeks of implantation and showed higher pin volume loss especially at 4 weeks after the operation in comparison to pure Mg and Mg2Ag (Figure 2A), which is consistent with the higher degradation rate (Figure 3). Consequently, the Mg10Gd implant showed a rather fast and uncontrolled degradation.

One explanation of the inhomogeneous degradation and faster disintegration performance of Mg10Gd, as we could show in our previous study [19], could be the Gd rich intermetallic particles that were found in the specimen's microstructure. The corrosion might occur on the grain boundaries and it propagated through the entire samples resulting in the failure/breakage of the pins. No particles were found for pure Mg and Mg2Ag showing a more homogeneous and moderate degradation during the 36 weeks of observation. This inhomogeneity in the degradation pattern of Mg10Gd continues till the end of the study.

Biological response within the bone

The biological response was investigated by a high resolution μ CT scan protocol related to the corresponding histological bone sections to analyze the implant integration in the bone tissue. Mechanical strength of the interface was assessed by push-out tests.

Pure Mg and Mg2Ag showed good integration into the femoral bone after 36 weeks. New bone was formed around the implant in the site of the intramedullary cavity. These materials degrade slowly and give the bone proper time for the adherence to the implant, bone formation around the implant surface and subsequently bone remodeling simultaneously with pin degradation. On the contrary, the disintegration of Mg10Gd in smaller particles during the study does not allow the normal healing and remodeling of the bone.

These results are comparable to a study performed by Kraus et al. [3] in which the fast degrading material ZX50 degraded within 24 weeks completely in comparison to a slower degradable material, WZ21. As a result of the high degradation rate of ZX50, a massive callus formation and release of high amounts of gas led to considerable bone tissue reactions. Although large amounts of hydrogen gas evolved within a short time period, the bone showed damage but was able to remodel completely within 24 weeks [3]. In contrast to our results of Mg10Gd, which has the fastest initial degradation velocity of the examined materials, no complete resolution of implant remnants takes place and no *restitutio ad integrum* can be

observed within 36 weeks. After the noticed disintegration into remnants, no further degradation could be observed for Mg10Gd.

Even though it is known [27] that large surface areas lead to higher degradation rates, no hints of further degradation like gas formation or decrease of the amount of degradation products were noticed for the large surface of remaining Mg10Gd. Possible explanations are (i) the high amount of bone formation around the particles, seen in the high resolution μ CT scans and histological staining, encapsulate the remnants and may reduce their dissolution capacity. This would be in accordance with Kraus et al., where reduced degradation capacity of WZ21 was observed in the cortical bone compared to the intramedullary cavity [3]. (ii) Cell types like macrophages are not able to phagocyte particles exceeding the size of 10 μ m [28] and therefore, the too large Mg10Gd remnants cannot be removed and degraded. (iii) Galli et al. also reported that Mg10Gd corrosion products stayed in their original shape [22]. Another possibility would be that these corrosion products form low-dissolvable remnants exhibiting a much slower degradation performance.

Clinical relevance and limitations of our study

In this study, pure Mg and the binary Mg-based alloys, Mg2Ag and Mg10Gd, are transcortically implanted in Sprague-Dawley® rats. Previous in vitro cytocompatibility studies indicated that Ag and Gd release do not affect the biocompatibility of these Mg alloys [19, 20, 26]. The results of our long-term in vivo study showed no signs of local toxicity; the implants were well integrated into the bone, and the bone layer surrounding the degradation products might indicate osteoconductivity of the implants. Also, no abnormal clinical observations were observed post operation.

Mg2Ag exhibited a preferable degradation rate within 36 weeks and was well integrated into the bone compared to pure Mg and Mg10Gd. Mg2Ag caused no adverse reactions like osteolysis or fibrotic encapsulation to the cortical bone during bone healing process which is promising regarding a possible clinical application.

Limitations of our study have to be taken into consideration. One limitation is the small sample size of $n=6$. Another limitation may be that degradation performance and bone response have been only investigated in a transcortical rat model and there is no knowledge whether and how these materials affect the growth plate neither in in vitro cell experiments (chondrocytes) nor in in vivo animal

studies (trans-epiphyseal growth plate animal model). As a consequence, conclusions regarding possible usage of these materials in children could not be pointed out from our study. For example, Pichler et al. have shown that WZ21 alloy which includes Y, exhibited promising cell-viability results in chondrocytes but there are still doubts about using this material for children because of Y toxicity [29]. One more limitation could be the duration of the study which ended at 36 weeks and no further conclusions could be drawn about full degradation of these materials. Blood examinations would have provided additional conclusions on materials safety.

Materials and methods

Magnesium alloys

Cylindrical pins of two binary Mg-based alloys (Mg2Ag, Mg10Gd) and pure Mg were used as testing devices. Pure materials Mg (99.99%), Gd (99.95%) and Ag (99.99%) were cast at Helmholtz-Zentrum (HZG), Geesthacht, Germany and prepared via extrusion, wire drawing and turning. The detailed production protocols have been described previously [19].

The chemical composition was determined by spark emission spectrometer (Spectrolab M, Spektro, Kleve, Germany). The contents of Ag and Gd were determined by X-ray fluorescence spectrometer (Bruker AXS S4 Explorer, Bruker AXS GmbH., Karlsruhe, Germany) (see Table 1) [19].

Animals, surgery procedure and euthanasia

All animal experiments were authorized by the Austrian Ministry of Science and Research (accreditation number BMWF-66.010/0078-II/3b/2011) and have been conducted on male Sprague-Dawley rats under ethical respects for animals. Forty-five male Sprague-Dawley® rats of 140–160 g weight and 5 weeks of age were used and randomly divided into three observation groups: (a) online μ CT group ($n=6$ animals/alloy) which underwent longitudinal μ CT scans at specific time points (1, 4, 12, 24 and 36 weeks); (b) histology group ($n=2$ animals/time point/alloy) euthanized after 4 and 24 weeks; and (c) push-out group ($n=3$ animals/alloy) euthanized after 4 weeks. Additionally, a

Table 1: Chemical composition of pure Mg, Mg2Ag and Mg10Gd pins were analyzed by using a spark emission spectrometer and a X-ray fluorescence spectrometer [19].

Alloy	Chemical composition wt. %					
	Ag	Gd	Fe	Cu	Ni	Mg
Pure Mg	–	–	0.0052	0.0023	0.0014	Bal.
Mg2Ag	1.75	–	0.002	0.0019	0.0013	Bal.
Mg10Gd	–	10.5	0.0029	0.0048	<0.0036	Bal.

sham group (n=6 animals) underwent transcortical drilling without pin implantation.

Cylindrical pins (1.6 mm diameter and 8 mm length) of pure Mg, Mg2Ag, and Mg10Gd alloys were implanted transcortically into the middle diaphysis of both femoral bones. The implantation surgery took place under general anesthesia. The whole surgical procedure, the postoperative treatment and euthanasia protocol have been described previously [3].

Micro-computed tomography scans

Longitudinal μ CT scans were performed at five different time points: 1, 4, 12, 24 and 36 weeks after the implantation with the Siemens Inveon micro CT (Siemens AG, Healthcare Sector, Erlangen, Germany). During μ CT scanning, the animals were anesthetized with volatile isoflurane (Forane®, Abbott AG, Baar, Switzerland). The Siemens Inveon Acquisition Workplace 1.2.2.2 (Siemens Healthcare GmbH, Erlangen, Germany) was the μ CT scan software which was used. In vivo μ CT scans of the rats were performed at 70 kV voltage, 500 μ A current, and 1000 ms exposure time. The effective pixel size was 35.04 μ m.

After euthanasia, the femoral bones were explanted and embedded in the Technovit 9100. Technovit blocks were scanned with a high resolution protocol in order to exhibit more details regarding the degradation performance and bone morphology during the bone healing process. High resolution μ CT scans were performed at 80 kV voltage, 500 μ A current and 1050 ms exposure time. The effective pixel size was 18.93 μ m.

Calculation of degradation rates

3-D morphometric analysis took place and the pin volume and surface and the gas volume were evaluated using Materialize Mimics® (Version 15.0 and 17.0, Materialize, Leuven, Belgium). For each time point n=6 bones were used. Following the measurement process of Kraus et al., pin volume, pin surface and gas formation were segmented from the μ CT data [3]. Implant degradation rates were calculated using the equation:

$$DR_i = \Delta V_i / \Delta t \text{ with } \Delta V_i = \Delta V_i / S_i$$

i=observation time point, ΔV_i =change of the volume between two observation time points (Δt) in mm^3 and S_i =surface area at the observation time point i in mm^2 [4].

Histological bone sections

For histology, the Technovit 9100 New (Technovit® 9100, Heraeus Kulzer, Frankfurt, Germany) embedding method was used as fully described by Willbold and Witte [30]. Bone explants were fixed in formalin for 48 h at 4 °C, dehydrated in different isopropanol degrees (70%, 96% and 100%) and in xylol at room temperature. The samples were infiltrated in pre-infiltration solution for 3 days at 4 °C and infiltration solution for 7 days at 4 °C. Afterwards, the bone samples were put into the embedding blocks and were covered with the polymerization solution. The polymerization procedure took place at

–4 °C for 5 days. After the polymerization, the tissue blocks were cut in 5 μ m with rotation microtome (HM 355S, Thermo Scientific, Histo-com, Vienna, Austria). The sections were dried for 2 days at 37 °C and stained with 0.1% Toluidine blue-O.

Push-out tests

The mechanical strength of the implant-bone interface at 4 weeks after implantation (n=6) was evaluated and quantified by push-out tests. Implanted pins were axially displaced in a controlled way under recordings of load and displacement. The energy needed to move the pin was calculated. The tests were performed at the TU Wien and the methodology was described in detail by Celarek et al. [31].

Statistical analysis

Results were analyzed using IBM SPSS Statistics 22 (SPSS: Version 22.0. Armonk, NY, USA). The results of pin volume, pin surface and gas volume between the 3 Mg alloys at each time point were analyzed by ANOVA one-way test. The significance level was set up on the p value ($p < 0.05$, $p < 0.01$ and $p < 0.001$). The results of the in vivo degradation rates were analyzed with the SPSS Mann-Whitney U test. The significance level was set up on the p value of 0.05.

Conclusion

The in vivo degradation performance of binary Mg alloys and pure magnesium were investigated with μ CT monitoring in a growing rat model in relation to the corresponding bone response. Two binary alloy systems, Mg2Ag and Mg10Gd, were chosen and compared with pure Mg in order to minimize the influence of multiple alloying elements on the degradation process. The following influences of the alloying elements can be concluded:

- (i) The alloying element Ag seems to slow down the degradation rate compared to pure Mg. On the contrary, the Gd element accelerates the degradation rate of the alloy compared to pure Mg.
- (ii) The Mg2Ag degrades slowly and more homogeneously whereas Mg10Gd disintegrates into small particles within 12 weeks post-operatively.
- (iii) The Mg2Ag pins showed no adverse reactions on bone healing process and remodeling. On the other hand, the Mg10Gd ion release seems to cause disturbances in bone healing process. The bone was not able to recover within the study period of 36 weeks and alterations were still present.
- (iv) The μ CT is, especially with its high resolution protocol, a suitable method to screen bioresorbable alloys during their degradation process. In combination with histological slices or also immunohistochemistry,

further information on bone reaction and biological response even at a cellular level can be analyzed.

These findings are considered as substantial significant in the development on Mg-based implant as medical device. Especially the unusual degradation behavior of Mg10Gd is planned for further studies to clarify the process within the organism and in what kind of state the remnants remain in the bone. Leading to further research, questions concentrating on toxicological studies of Ag and Gd distribution in tissues and at the implantation site to reveal the metabolism within the organism are of high importance. Additionally, sort of cell types which surround the implant degradation products and whether the remnants will be completely degradable should be further investigated.

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Author's statement

Conflict of interest: Authors state no conflict of interest.

Materials and methods

Informed consent: Informed consent has been obtained from all individuals included in this study.

Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the authors' institutional review board or equivalent committee.

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