Stiffness of metals, alloys and components

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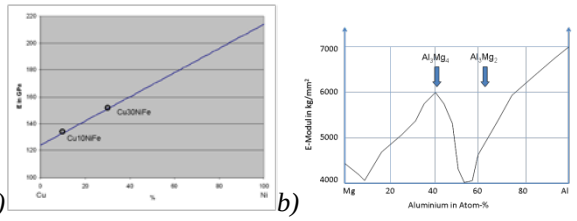
INTRODUCTION: Stiffness is load divided by deformation and often declared as an important factor for the design of metallic components in engineering as well as for the design of implants. It can also be defined as the rigidity of an object - the extent to which it resists deformation in response to an applied force [1]. It is also important to understand that stiffness is not strength and that engineers and clinicians are talking about the same matter.

STIFFNESS OF MATERIALS: Elastic constants like the Young's (E), shear (K), or bulk (G) moduluscan be seen asthe intrinsic stiffness of a material under tension, shear and compression.These constants basically reflect the bonding strength between atoms as long as the material is loaded in the elastic region.Due to the fact that metals are polycrystalline and all crystals contain numerous defects, the theoretical strength of a metal/alloy can also never be reached.



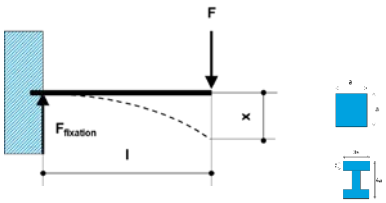
a) *b)*

Fig.1: Development of E in a)Cu-Ni[2,3], Mg-Al[4]

However, alloying, deformation and the appearance of intermetallic phases definitely changes elastic constants. Figure *1*a) shows exemplarily the development of E for the binary Cu-Ni system. Both have the same crystal lattice structure, similar lattice constants and the melting points are not too different. As a result E follows a linear rule of mixture with respect to the increase of Ni in Cu.Figure *1*b) shows the development of E for Mg-Al which forms intermetallic phases. In this case the amount of intermetallic phases influences how E develops.

The first case is not applicable to Mg alloys due to the fact that there is no known system of complete solid solubility. Most binary Mg-X systems are eutectic at the Mg rich side, a few show peritectic reactions. However, a certain amount of intermetallic phases forms during solidification or subsequent heat treatments and can be used to alter/tailor E.

STIFFNESS OF COMPONENTS: Let us take a simple beam and its deflectionx (figure *2*)as an example how stiffness is influenced by the geometry of an objectmade out of Mg or Fewith different E. The geometry of the beam is quadratic and I-shaped and the load F (1N) and length (1m) are constant. The deflection can be calculated using equation 1 which shows the influence of E and the area moment of inertia I_x (equations 2 and 3).



$$x = \frac{Fl^3}{3EI_x} \quad (1)$$

$$I_x = \frac{a^4}{12} \quad (2)$$

$$I_x = \frac{44a^4}{3} \quad (3)$$

Fig. 2: Deflection x of a beam(length l) with a constant load F and area moments of inertia for two different geometries of the beam

With increasing Ethe deflection decreases. But the magnitude of E can also be compensated with the modification of I_x i.e. the geometry of the beam. When comparing Mg and Fe this would mean that when $I_{x,Mg}$ is 4.67 larger than that of Fe the deflection of both beams would be equal (equal stiffness).When for a chosen material the area of cross section of the beam would be kept constant, the I-beam would have an I_x that is higher by a factor of 2.75 compared to a quadratic cross section. This means that the I-beam 2.75 times stiffer than the beam with a quadratic cross section.

CONCLUSIONS: Stiffness is composed of the intrinsic stiffness of materials (matrix + intermetallics/reinforcements) and the chosen geometry of a component. Therefore the stiffness of a material can be tailored by composition and/or geometry.

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Cold-drawn ZM21 and WE43 wires exhibit exceptional strength and ductility

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INTRODUCTION: Cold drawn wire is extensively used in the production of medical devices. In addition to being a very efficient processing method, cold drawing allows exceptional grain refinement and work-hardening, excellent dimensional control and surface finish, and facilitates many existing subsequent device fabrication steps.

However, cold drawn magnesium alloys have not been produced on a commercial scale. Applying cold drawing techniques to magnesium alloys presents significant challenges due to limited deformation mechanisms available at room temperature. However, recent reports would indicate the feasibility of HCP magnesium alloys with moderate levels of cold work [1-3].

The aim of this work was to investigate the possible reduction and work hardening in two commercially available HCP alloys, ZM21 and WE43.

METHODS: Extruded ZM21 (Magnesium Elektron, Manchester, UK) and WE43B (Alloys International, Islandia, NY, USA) materials were obtained. These were cold drawn and subsequently annealed by conventional methods to reduce the wire diameter. This draw-anneal cycle was repeated as necessary until a final anneal at a diameter of 510 µm.

The annealed wires were then cold drawn until failure to determine maximum reduction, with samples taken for tensile testing. Cold-worked samples were then subjected to various thermal treatments to determine aging response.

RESULTS: ZM21 was drawn to a diameter of 100 µm (true strain 3.2). UTS increased from 200 MPa annealed to 412 MPa at the highest level of cold work. This was accompanied by a loss in uniform elongation from 10.4% to 3.4% (Figure 1).

WE43 was cold drawn to a diameter of 71 µm (true strain 3.94). UTS increased from 267 MPa annealed to 565 MPa at the highest level of cold work. Elongation decreased from 15% to 1.8%. Aging drawn WE43 at 250C increased UTS to 640 MPa (Figure 2).

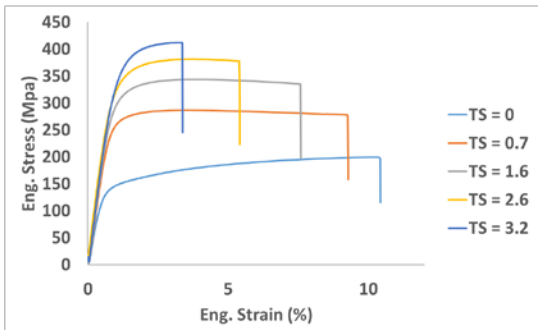

Fig. 1: Tensile curves for ZM21 wires.

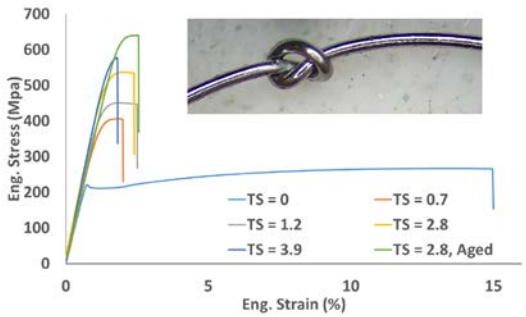

Fig. 2: WE43 wire drawn to various levels of true strain and aged. Image is 127µm wire with TS=2.8 and knotted closed, indicating good formability.

DISCUSSION & CONCLUSIONS: The levels of cold reduction achieved are greater than 3 times those previously reported [1], and the strength increases give the highest reported alloy-specific strengths. The strengths achieved by the aged WE43 are the highest tensile strengths reported for any crystalline Mg alloy. The drop in uniform tensile elongation with increasing cold work is significant, and may limit the utility of the wire in some applications such as balloon-expandable stents, while enabling self-expandable Mg stents.

The successes here with two very different alloys would indicate the potential of cold-drawing a wide range of Mg alloys, including those specifically designed for biomedical applications.

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In situ synchrotron radiation diffraction during solidification of Mg15Gd

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INTRODUCTION: Mg-Gd alloys have potential to be used for bio-applications as degradable implant material [1]. Casting plays an important role during alloy development: Phase formation and microstructure evolution are at first determined during solidification. *In situ* synchrotron radiation diffraction is a unique tool to follow phase formation, phase transformations and grain growth during solidification. The solidification sequences obtained with different cooling rate are of interest.

MATERIAL & METHODS: Pure Mg (99.95%) and Gd (99.98%) were used to cast the Mg15Gd by permanent mould direct chill casting [2]. Samples for solidification experiements were machined from the as-cast ingot and contained in graphite crucibles (d = 4 mm, h = 3 mm) . The measurements were performed at the P07 (HEMS) Beamline of PETRA III at DESY. The beam energy was set to 100 keV (λ = 0.0124 nm), the acquisition time was 0.1 s, 10 images were summed to obtain the Debye-Scherrer patterns. The measurement was performed in the chamber of a DIL 805A/D dilatometer in Ar flow. The samples were heated to 750 °C and held for 5 min, to ensure melt homogeneity then cooled to 200 °C with a cooling rate of 20 K/min which was controlled by a type S thermocouple. The information on the intermetallic phases were obtained from the Pearson's Crystal Structure Database [3], the d-spacings and 2□angles for the phases were calculated using CaRIn Crystallography 3.1™ software.

RESULTS: The line profiles calcualted from the azimuthal integration of the 2D diffraction patterns are shown in Figure 1. The solidification sequence starts with the formation of the α-Mg phase at 640°C. As the cooling advances the Mg peaks become more pronounced and at 561°C the formation of the Mg_3Gd can be observed which transforms into the Mg_5Gd at 551°C. The results were correlated with thermodynamic simulations conducted with the Pandat 8.1 software and a database PanMagnesium8. The Scheil calculation predicted the α-Mg at 627°C and Mg_5Gd at 576°C.

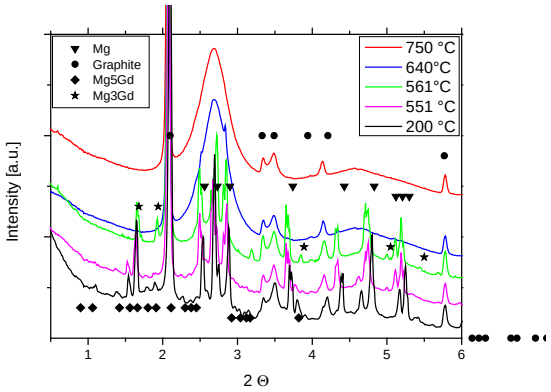

Fig.1: Sequence of XRD line profiles during solidification of Mg15Gd.

DISCUSSION & CONCLUSIONS: During the cooling at 20 K/min from 750°C the solidification sequence of Mg15Gd was determined experimentally and correlated with thermodynamic calculations. The solidification of α-Mg started at 640°C, higher than the predicted 627°C, and the formation of the metastable secondary phase, Mg_3Gd started lower at 561°C than the predicted formation of Mg_5Gd at 576°C. The formation of Mg_5Gd occurs at 551°C, while at the same temperature the Mg_3Gd phase disappears. Although the thermodynamic simulations could not predict the formation and transformation of the metastable Mg_3Gd, it could be shown experimentally. The results can improve the existing databases within the measurement accuracy (±3°C).

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Investigation on Zinc based alloys for biodegradable stent applications

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INTRODUCTION: Biodegradable metallic stents are used as stabilizing scaffolds to maintain the patency of the vessel and avoiding restenosis. Recently, several studies have been focused on Fe alloys and Mg alloys as biodegradable stent materials. However, it is proved that pure Fe has a low degradation rate and accumulation of corrosion products in an arterial wall causes a critical threat for the integrity of the arterial wall. Besides, Mg corrodes too fast in a physiological environment, leading to high hydrogen evolution rate. Accordingly, the two mentioned materials are not fully appropriate for coronary stent applications. Zinc is known as one of the essential elements for the human body. Since the corrosion rate of Zn is between Fe and Mg it can potentially be suitable for stent applications, however, pure zinc exhibits poor mechanical properties. The mechanical and corrosion properties of Zn-based alloys compared to those of pure Zn and Mg were investigated.

METHODS: Zn-0.5Al and Zn-0.15Mg were prepared by melting pure Zn (99.995%), Al (99.995%) and Mg (99.95%) in a resistance furnace at 500°C. Homogenization was insured by mechanical shaking of the crucible. Microstructural characterization was performed by optical microscopy. Mechanical properties were characterized by Vickers micro-hardness test. The corrosion behaviour of the alloys was compared to that of pure Zn and Mg by performing potentiodynamic polarization (1 mVs^{-1}) tests in phosphate buffered solution (PBS) of pH 7.4 and controlled temperature of 37°C.

RESULTS: Fig.1 shows microstructures of the investigated materials. As seen, the pure Zn possesses quite large grains, however, after alloying with 0.5 wt.% Al, grain size was reduced to about 150 µm. Moreover, due to the rapid solidification, the alloy had a supersaturated single phase microstructure. In contrast, Due to low solubility of Mg in Zn, Zn-Mg alloy consisted of Zn-rich dendrites accompanied by eutectic along the grain boundaries.

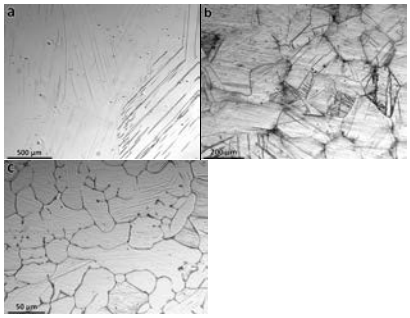

Fig. 1: Microstructure of the investigated alloys: pure Zn (a), Zn-0.5Al (b), and Zn-0.15Mg (c).

As shown in Table. 1, by adding Al and Mg the micro-hardness values of the alloys were markedly improved to 59 and 79 HV, respectively.

Table 1. Micro-hardness of the specimens.

Samples	Mg	Zn	Zn-0.5Al	Zn-0.15Mg
Micro-hardness [HV]	33±2	31±1	59±3.5	79±3

The corrosion parameters obtained from the investigated samples are summarized in Table 4. The corrosion rates of all of the Zn alloys are significantly lower than that of Mg. Moreover, it is observed that the effect of Mg and of Al on the corrosion rate of the Zn–Mg alloys is small.

Table. 2: Corrosion parameter of the materials obtained from the potentiodynamic curves.

Samples	Mg	Zn	Zn-0.5Al	Zn-0.15Mg
J (µA cm^{-2})	44.7	7.5	7.2	7.3
E (V)	-1.5	-0.975	-1.025	-1.045

DISCUSSION & CONCLUSIONS: Zn based alloys with small amount of different alloying elements were prepared by melting-casting method. Microstructural observation revealed that by adding Al and Mg to Zn, the average grain size was reduced in both alloys. The micro-hardness increased by adding the above elements. Regardless of the small amount, Mg seems to be more effective in improving mechanical strength of the alloy due to the formation of the eutectic phase. The prepared alloys showed remarkable corrosion properties compared to those of Mg, more importantly, since the amount of added elements was quite low, the differences in corrosion rate of the Zn based alloys were almost negligible.

In vitro study on novel Zn-ZnO composites with tunable degradation rate

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INTRODUCTION: Currently, the widely investigated Mg, Fe and their alloys are facing the corrosion rate problems, either too fast or too slow. Zn has standard electrode potentials between Mg and Fe, implying a more apposite degradation rate. Moreover, Zn is an essential element of human body as well. In the present study, Zn composited with different content of ZnO was studied to see if we can tune its degradation rate.

METHODS: Pure zinc powder and zinc oxide powder were used as raw materials to fabricate Zn-XZnO (X=0.25, 0.5, 1 wt.%) composites. via a SPS-1050 system. Optical microscopy and XRD were conducted to reveal the microstructure and constituent phases. Mechanical properties were studied by compression and microhardness testing. The corrosion properties were characterized by immersion test and electrochemical measurement. SEM equipped with an EDS was used to analyse the corrosion products. L929 and ECV304 were adopted to probe the cytotoxicity of specimens with both direct and indirect methods.

RESULTS: Mechanical properties of studied material were listed in *Fig. 1.* The addition of second phases deteriorated both the ultimate compression strength (UCS) and yield strength (YS) of pure zinc slightly. The microhardness of the composites altered a little with different contents of ZnO. Electrochemical parameters were listed in *Table. 1*. Zn-XZnO composites displayed higher corrosion current densities than pure zinc which meant faster degradation rates. Immersion test implied the same results and from EDS analyses it could be assumed that corrosion products contained ZnO and CaP compounds. The extracts of Zn-XZnO (X=0.25, 0.5, 1 wt.%) composites demonstrated insignificant cytotoxicity to ECV304 cells after 1, 3, 5 days culture, while showed mild cytotoxicity to MG63 cells.

DISCUSSION & CONCLUSIONS: Compared with pure zinc, Zn-XZnO composites with ZnO content below 1wt.% exhibited similar mechanical properties and more appropriate degradation rates, especially for the Zn-0.5ZnO. In conclusion, compositing Zn with ZnO is a promising method to adjust the degradation rate of Zn, and other materials need to be further tried as reinforce phases in consideration of mechanical property.

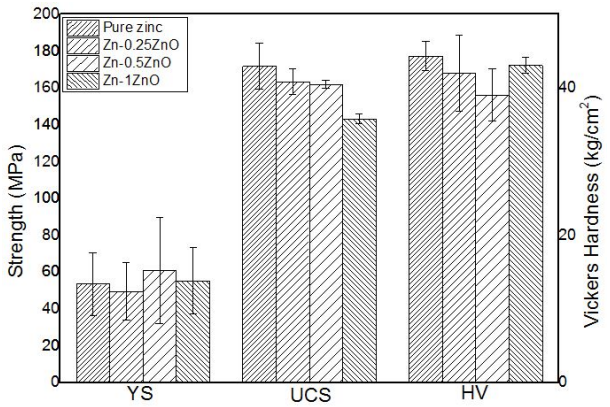

Fig. 1: Mechanical properties of studied materials.

Table. 1: Average electrochemical parameters of Zn-XZnO composites. (CR: Corrosion Rate; EC: Electrochemical; I: Immersion)

	i_{corr}	v_{corr}	*CR* g/(m².d)	
	μA/cm²	mm/year	*EC*	*I*
Pure zinc	0.528	0.008	0.154	0.024
Zn-0.25ZnO	7.246	0.108	2.107	0.186
Zn-0.5ZnO	6.275	0.093	1.823	-0.156
Zn-1ZnO	0.599	0.009	0.174	0.5

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Experiments and numerical simulations to evaluate peeling properties of polymeric coatings for degradable Mg stents

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INTRODUCTION: Biodegradable stents attracted the attention of many researchers since they can absolve their specific function for the expected period of time and then gradually degrade. Up to now biodegradable stents made of polymers or magnesium (Mg) alloys have been proposed. However, both the solutions have limitations. The polymeric stent have limited mechanical properties and barely withstand the natural contraction of blood vessels. The magnesium stents dissolve too fast in the human body. Our past studies were focused on: i) the selection of a Mg alloy suitable for stent production, having sufficient strength and elongation capability; ii) the optimization of the stent geometry to minimize stress and strain after stent deployment and improve scaffolding ability; iii) the selection of a polymeric coating able to assure enough corrosion resistance; iv) laser cutting and surface finishing of Mg stents. In this work we present some results of an experimental and numerical ongoing study aiming to the development of biodegradable stents made of Mg alloy coated with a degradable polymer with an improved corrosion resistance.

METHODS: PCL (poly-caprolactone, Mn = 80 000 g/mol, Sigma-Aldrich, product number 440744-250G) coatings were obtained by a dipping procedure. PCL was dissolved in chloroform at a concentration of 5% w/v and was dropped onto AZ31 samples (70mm x 4mm x 2mm). Samples were left under a hood for 24 hours to allow the complete solvent evaporation and a uniform PCL coating with a thickness of 0.01 mm was obtained. Finite element analyses (FEA) with cohesive zone method were carried out with the commercial code ABAQUS (Dassault Systèmes, Simulia Corp., USA). Experimental tests were performed to find the cohesive element parameters to be used in the model. A 90-degree peeling test (Fig. 1 left) was carried out on a MTS Synergie 200H testing machine (MTS Systems Corporation, Minneapolis, MN,USA) with a 100 N load cell. Test was performed in triplicate. The experimental tests were reproduced numerically by FEA (Fig.1 right). Subsequently, a 2D stent strut model with a polymer coating was extracted from a 3D stent model previously developed (Fig.2 left). A symmetrical boundary condition in Y-direction was applied to one strut end and a displacement in Y-direction was applied to the other end to simulate the stent expansion to a final diameter of 3 mm. After expansion, the cohesive elements were stretched and the coating had different separation ranges according to the location.

RESULTS: None of the 2D stent strut simulations reached the tensile limit stress obtained in the peeling experimental tests; hence, the estimated coating damage was 0 as reported in Fig. 2 (right).

DISCUSSION & CONCLUSIONS: The simulations suggest that under the studied conditions the peeling should not occur to the coating of the investigated stent design. Tests are under investigation to verify our numerical findings. Such a model might be used to optimize the design of a degradable coated stent.

ACKNOWLEDGEMENTS: Wei Wu is supported by the Politecnico di Milano International Fellowships Program.

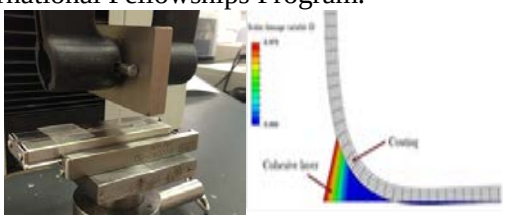

Fig. 1: Experimental (left) and numerical (right) peeling test on AZ31 sample coated with PCL.

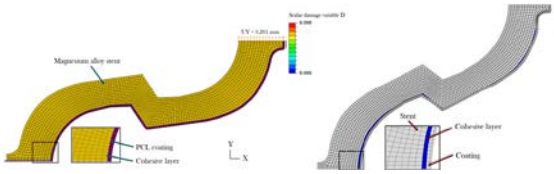

Fig. 2. FEA 2D model of a coated stent strut (left) and final configuration after stent expansion with results in terms of damage parameter in the coating (right).

Binder-jetting 3D Printing (3DP) of Customized Patient Specific Craniofacial Scaffolds of Fe-Mn based Alloys

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INTRODUCTION: Fe-Mn was introduced as a potential biodegradable metal candidate albeit displaying slow degradation, but higher mechanical strength and anti-ferromagnetic properties [1]. Binder-jetting 3D printing (3DP) of the alloy for fabricating porous medical scaffolds exhibiting higher degradation has also been demonstrated [2]. Current work has explored the biocorrosion and cytotoxicity of modified Fe-Mn powder alloys using high energy mechanical alloying (HEMA) and cold isostatic pressing (CIP). In addition, the Fe-Mn-M[1] alloy was selected for binder-jetting 3D printing (3DP) and investigated further to study the density, open porosity, and compressive mechanical properties in comparison to 3DP of pure Fe and pure Fe-Mn alloys.

METHODS: Pure elemental Fe, Mn, and M1 powder in high purity were mechanically alloyed using P5 planetary milling machine. Fe-Mn, and Fe-Mn-M1 powder alloys were consolidated using cold isostatic press and sintered under a gettered ultra-high purity argon environment. Sintered alloy specimens were machined for bio-corrosion and cell cytotoxicity assessment. Potentiodynamic polarization (PDP) and live/dead assay were performed to select the candidate material for binder-jetting 3D printing. Fe-Mn-1M[1] composition was therefore studied for binder-jetting 3D printing using ExOne ProMetal RxD printer. 3D-printed specimens were sintered and characterized for density, open porosity, and compressive mechanical properties. The surface morphology of sintered 3DP samples were imaged using scanning electron microscopy.

RESULTS: Fe-Mn-1M[1] pellet exhibited higher cytocompatibility determined by the higher cell count from the fluorescent images of live/dead cell viability assay after 1 and 3 days of culture. PDP and live/dead cell viability assay exhibited the enhanced degradation of Fe-Mn system without deteriorating the cell viability by addition of M1 (see Table 1 and Fig. 1).

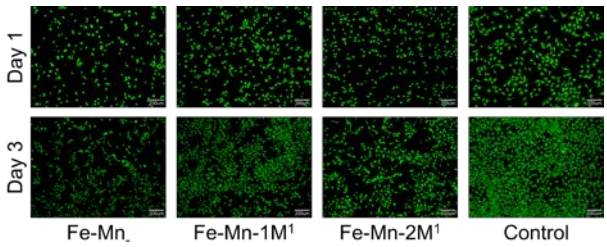

Fig. 1: Live/dead cell viability assay images of Fe-Mn and Fe-Mn-M[1] specimens.

Table 1: Corrosion potential and current density of Fe-Mn based pellets from PDP measurement

Material	Corrosion potential, E_{corr} (V)	Corrosion current density i_{corr} (μA cm^{-2})
Fe-Mn	-0.447	1.65
Fe-Mn-1M[1]	-0.574	1.77
Fe-Mn-2M[1]	-0.659	14.3

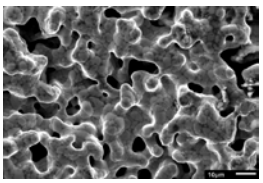

Fig. 2: SEM Images of the surface of as-sintered 3DP Fe-Mn-1M[1]

3DP Fe-Mn-1M[1] showed a total density (6.86 g/cc), envelop density (3.38 g/cc), and 50.8% open porosity. Compression tests yielded UTS of 425.4 MPa for 3DP Fe-Mn-1M[1] alloys.

DISCUSSION & CONCLUSIONS: The current research demonstrates the enhanced degradation of modified Fe-Mn-M[1] alloys as well as binder-jetting 3DP of these alloys to construct a porous scaffold with suitable mechanical strength.

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Effect of Sc addition on microstructure, mechanical properties and *in vitro* corrosion behaviour of biodegradable Mg-1.5Zn-0.6Zr (ZK21) alloy

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INTRODUCTION: To avoid intensive galvanic corrosion reactions, we have developed a single-phase Mg-1.5Zn-0.6Zr (ZK21) alloy and the results show that the ZK21 exhibits perfect corrosion behaviour. Literature demonstrates that addition of rare earth elements (REEs) could enhance the corrosion resistance of magnesium alloys. Although REEs may display harmfulness to human body [1], as the lightest REE scandium (Sc) could be safe. It is quite a pity that Sc addition to magnesium alloy is seldom studied. In this work, the effect of Sc addition on microstructure, mechanical properties and *in vitro* corrosion behaviour of the ZK21 alloy were investigated.

METHODS: ZK21-*x*Sc (Zn 1.5wt%, Zr 0.6wt%, *x* = 0, 0.2, 0.5, 1.0wt%) alloys were cast. Vickers hardness (H_v) and friction coefficient (μ) of these alloys were tested. *In vitro* immersion tests were conducted at 37±0.5 °C in Hank's solution, with a pH value of 7.4, for 168 h. The ratio of solution volume to specimen surface area was 30 ml/cm^2. The generation of hydrogen gas and variation of pH value in Hank's solution were monitored during the immersion test. Electrochemical impedance spectroscopy was measured in Hank's solution at 37 °C. A three-electrode electrochemical cell was used. The measurement was performed at open circuit potential with an AC amplitude of 10 mV over a frequency range from 1 MHz down to 100 mHz.

RESULTS: Table 1 shows the tested Vickers hardness and friction coefficient of ZK21-*x*Sc alloys. The Vickers hardness gets higher with increasing Sc content. While for the friction coefficient, the situation was the opposite. Fig. 1 shows the generation of hydrogen gas and variation of pH value of Hank's solution during immersion. Both the hydrogen gas volumes and pH values show the same tendency in the order of ZK21-0.2Sc < ZK21 < AZ91D < ZK21-0.5Sc < ZK21-1.0Sc < Pure Mg. The results imply that ZK21-0.2Sc has the lowest corrosion rate. Fig. 2 shows the corrosion rates determined by weight loss and the Nyquist curves of alloys immersed in Hank's solution. The corrosion rate of ZK21-0.2Sc was 0.3270 mm/y, the lowest among all the alloys. The Nyquist curves confirmed that.

Table 1. Vickers hardness and friction coefficient of ZK21-xSc alloys.

x	0	0.2	0.5	1.0
H_v	53.68	56.28	57.66	60.82
μ	0.1324	0.1316	0.1304	0.1268

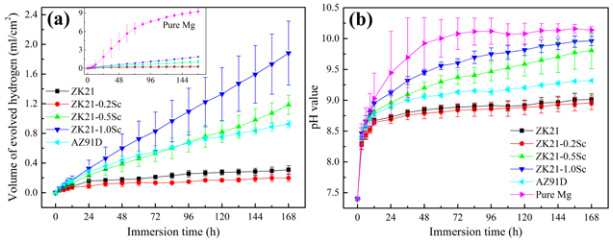

Fig. 1: (a) Generation of hydrogen gas. (b) Variation of pH value of Hank's solution.

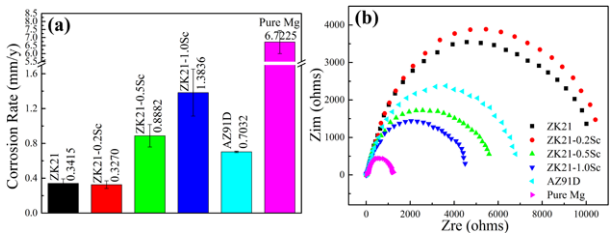

Fig. 2: (a) Corrosion rates determined by weight loss. (b) Nyquist curves of alloys immersed in Hank's solution.

DISCUSSION & CONCLUSIONS: The addition of Sc can strengthen the ZK21 alloy. The ZK21-0.2Sc shows the best corrosion behaviour. The addition of 0.2% Sc can reduce the corrosion rate of ZK21, while further increasing Sc content could form precipitation particles that induce the galvanic corrosion and therefore deteriorates the corrosion behaviour.

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ACKNOWLEDGEMENTS: This project was supported by National Natural Science Foundation of China (No. 51174025).

Innovative biodegradable Zinc-based alloys for load-bearing biomedical implant applications

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INTRODUCTION: Biodegradable implants offer several advantages in biomedical implant applications over their non-degradable counterparts used today. Prime among them is the promise that new implants based on these materials will eventually dissolve when they are no longer needed, hence eliminating residual implants or the pain and expense of a second removal surgery that could be required otherwise.

METHODS: Biodegradable metals are more suitable for load-bearing biodegradable implants due to their superior mechanical properties compared to polymeric materials. From biological aspect, released zinc ions from Zn alloy implants degradation may exemplify many of the advantages gained by using biodegradable orthopedic implants and coronary stents. The main advantage of Zn alloy over Mg alloy is its high corrosion resistance in body fluid environment (see Table 1), considering current Mg alloy implants often suffer from fast-corrosion and consequent release of hydrogen bubble and early dislocation and disruption. *In vitro* and *In vivo* degradation tests showed that degradation rate of Zn alloy is much lower than that of Mg alloy, and it can reach 6 to 12 months long, and which is adjustable. Tensile test revealed that Zn alloy had superior mechanical properties, such as 430 Mpa of tensile strength and 21% of elongation.

RESULTS: In vitro cytotoxicity test results indicated that Zn alloys were biocompatible since cells growing in contact with corrosion products of Zn alloys maintained high cell viability and healthy morphology. Preliminary animal study demonstrated that Zn alloys had excellent biocompatibility and no detrimental corrosion products were found in surrounding tissue. Biodegradable stent and screws & pins were fabricated using our new developed Zinc alloy, see Figure 1 and Figure 2.

*Tab 1. Comparison of corrosion rates (mg/cm^2*h)*

	2 weeks	4 weeks
Zn-1Mg	0.014	0.0058
WE43	0.038	0.028

Fig. 1: Zinc alloy made coronary stent with diameter of 2 mm and wall thickness 0.15 mm.

Fig. 2: Our manufactured Zinc alloy biodegradable interference screws (left 2) and transfix pins (right 2) used in ACL.

DISCUSSION & CONCLUSIONS: We truly believed that our specially manufactured Zinc alloy with homogenous microstructure, uniform and slow biodegradation, superior mechanical properties and excellent biocompatibility is a potential candidate material for load-bearing biodegradable implant applicationss should be set as one block, as below, and a maximum of four references may be used.

The prospects of EW62 Mg alloy as a structural material for biodegradable implants

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INTRODUCTION: Mg alloys demonstrate attractive properties as a structural material for biodegradable implants in term of their biocompatibility and deterioration characteristics [1]. However, Mg implants exhibit accelerated corrosion in physiological environments which results in premature loss of mechanical integrity, hydrogen gas evolution and separation of tissues [2]. In order to reduce the accelerated corrosion problem a new Mg alloy: Mg-6%Nd-2%Y-0.5%Zr (EW62) was developed in the form of conventionally cast ingots (CC) and rapidly solidified bars (RS). The prospects of CC and RS systems to serve as biodegradable implants were evaluated in terms of their corrosion and stress corrosion resistance.

METHODS: Ingots of EW62 Mg alloy were prepared by melting Nd, Zr and Y in pure Mg. The melting process was carried out in a carbon steel crucible under protective gas atmosphere of CO_2+HFC134. The molten alloy was cast in a steel mold to obtain 8 kg ingots. By re-melting the cast ingots at 750°C, rapidly solidified ribbons were produced under an Ar atmosphere using a single-roller melt-spinning set-up. The ribbons with a width of 8 mm and a thickness of 120 µm were then compressed into a cylindrical shape, 50 mm in diameter and extruded at 400°C into cylindrical bars of 10 mm diameter. The in-vitro corrosion behaviour of EW62 alloy in the form of CC ingots and RS bars was evaluated in saline solution saturated with $Mg(OH)_2$ at 37°C for immersion test and stress corrosion analysis by slow strain rate tensile (SSRT) testing. The surface characteristics of CC and RS were examined by SEM and XPS.

RESULTS: The mechanical properties of RS alloy compared to CC alloy were significantly improved. The ultimate tensile strength of RS alloy was 274 MPa and elongation to failure was 16%. The stress corrosion resistance of RS alloy was also relatively improved even at low strain rates in terms of UTS and elongation to failure. The corrosion rate obtained by immersion test for RS alloy after 1 day was 0.5 mm/year and after 10 days stabilized at 0.07 mm/year compare to that of the CC alloy: 2.1 mm/year, and 0.26 mm/year after 1 and 10 days, respectively. The improved corrosion resistance of the RS alloy compare to the CC alloy after immersion for 5 days in terms of surface appearance is shown in Fig. 1.

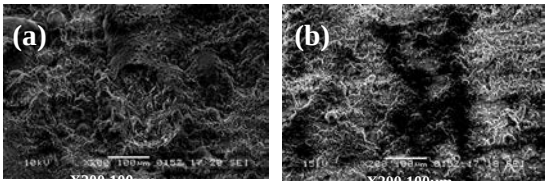

Fig. 1: SEM of the corroded surface of EW62 alloys after immersion in saline solution saturated with $Mg(OH)_2$ at 37°C for 5 days.(a) CC alloy, (b) RS alloy.

The X-ray photoelectron spectroscopy analysis of the corroded surface revealed a dominant presence of Nd_2O_3 with some MgO in the external layer up to a depth of 80 nm, compare to the composition of mainly MgO with some Nd_2O_3 up to the depth of only 20 nm in the external layer of the CC alloy.

DISCUSSION & CONCLUSIONS: The improved corrosion behavior and stress corrosion resistance of the RS alloy compare to that of the CC alloy is mainly attributed to the improved microstructure properties in terms of homogeneity and characteristics of the external scale. The relatively significant enrichment of the external MgO layer with Nd_2O_3 in the case of the RS alloy played a vital role in improving its relative corrosion resistance. Hence it is believed that EW62 alloy in the form of rapidly solidified structure have increased potential to reduce the accelerated corrosion of Mg base implants.

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Comparative studies of mechanical, corrosion and cytotoxity of MgZnCa and MgZnCa-REs alloys

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INTRODUCTION: Mg-based alloys have been anticipated to be very promising implant materials for both orthopaedic and cardiovascular applications [1]. They have to possess good corrosion resistance and good mechanical strength and ductility usually by alloying. One common way to control the cytotoxicity is to use non-toxic essential elements, such as Zn, Ca, etc. and low-toxic elements, such as heavy rare earth (RE) elements (Gd, Dy, etc.). Zn, Ca and REs had shown strengthening ability and improvement in corrosion resistance of magnesium alloys. In this paper, a study to compare the mechanical, corrosion and cytotoxity performance of two closely related alloys systems (MgZnCa and MgZnCa-REs) are to be reported.

METHODS: Two alloys systems with nominal compositions of Mg-4wt.%Zn-0.3wt.%Ca, designated as alloy-1, and Mg-1.0wt.%Zn-0.3wt.%Ca-1.3wt.%REs, designated as alloys-2 were melted and casted in a low carbon steel crucible in an inert argon environment. The casting temperature was 750ºC. Microstructures were observed on polished and etched surfaces by optical microscope. Phases were further examined with scanning electron microscope and energy dispersive X-ray spectrometry (EDS). Mechanical tests were performed in accordance of ASTM E8/E8M-11. As-cast and T4 treated materials were used for corrosion and biological tests. Corrosion property was determined by immersion tests. The MC3T3-E1 pre-osteoblast cell line was adopted for the cell viability assessment. Live/dead assay and MTT assay were performed as in ref. [2].

RESULTS: Mechanical property, corrosion rate are listed in Table 1. In-direct live/dead assay ISO (10993-5) morphological gradings for alloy-1 in as-cast, T4 2h and T4 8h at 100% extract was 0-1, 1-2 and 0-1, respectively. For alloy-2 in both as-cast and T4 condition at 100% extract, the grading was 0-1. This set of data shows alloy-1 in T4 2h condition has a slight cytotoxicity. Fig.1. shows the MTT assay results for the two alloys. It is indicated the alloy-2 has better metabolic cell activity than alloy-1.

Table 1. Materials property.

Alloys	YS (MPa)	UTS (MPa)	Elongation (%)	Corrosion rate (mm/y)*
Alloy-1 as-cast	67.9	199.1	9.3	4.6
Alloy-1 T4				20
Alloy-2 as cast	121.0	232.7	11.3	2.4
Alloy-2 T4				0.45

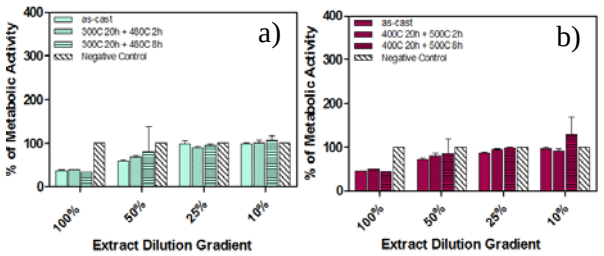

Fig. 1. Average percent of metabolic activity of MC3T3 cells cultured under extract dilutions for 72 hours for as-cast and heat treated a) alloy-1 and b) alloy-2.

DISCUSSION & CONCLUSIONS: Though alloy-2 has only total 0.82at.% of alloying elements compared to 1.69 in alloy-1, its outstanding mechanical property shows the advantage of REs. RE-containing alloy-2 has much better corrosion resistance, which explains the better cell culture performance.

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Benefits and limitations of biodegradable Mg implants and parts, produced by MIM (Metal Injection Moulding)

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INTRODUCTION: Biodegradable implants made of polylactide acids (PLA) and polyglycolic acids (PGA) are currently in common clinical use. These polymer products manufactured by injection moulding technique suffer from poor mechanical properties, acidic degradation behaviour and degradation times between 24 to 36 months [1]. Looking to the future, these polymer based implants might be substituted by magnesium based parts, produced by a similar technique, namely metal injection moulding (MIM). In comparison to polymers, biodegradable Mg based implants produced by MIM provide higher mechanical strength (Mg-0.9Ca: UTS=131 MPa), an elastic modulus of 30-40 GPa, alkaline degradation behaviour [2] and shorter degradation times. This study focusses on benefits and limitations of MIM processing of Mg based parts and implants.

METHODS: Pure Mg powder (SFM-SA, Switzerland) and Ca rich MAP (Master Alloy Powder) (ZfW-Clausthal, Germany) were used for mixing Mg-0.9Ca powder blends. For injection moulding, additional polymer components were added (PP-co1-PB, wax, stearic acid). Injection moulding took place on a commercial Arburg Allrounder 320S machine. Solvent debinding was performed in hexane at 45 °C for 10 h (Lömi, Germany,) and sintering was done between 635-645 °C for 8-64 h under Argon atmosphere. The detailed process is described elsewhere [3-4]. The microstructure was studied using light microscopy (Olympus PGM 3) and SEM (Zeiss DSM 962). Photoshop- and analySYS pro software as well as Archimedes method were used to investigate the porosity. E-moduli were determined by using resonant ultrasonic spectroscopy (RUS), (RFDA, IMCE, Belgium). Materials testing were carried out by tensile- and compression tests on a Zwick Z005 machine.

RESULTS: The whole MIM processing route, starting from green part production, debinding and final consolidation by sintering was performed successfully. Two major process variations, influencing the sintering results, were identified:

- Polymer content in the Mg feedstock
- Furnace and atmosphere conditions

Figure 1 shows how porosity and elastic modulus can be adjusted by these parameters. It also shows the linear ratio between Young´s modulus and porosity.

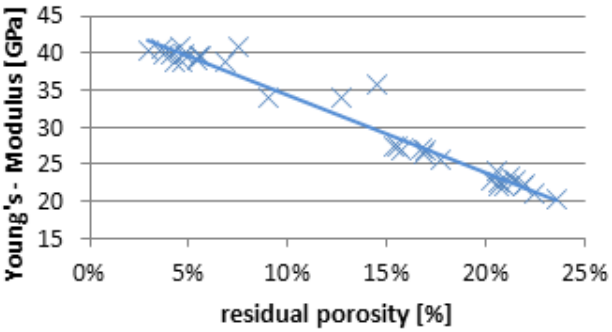

Fig. 1: ratio between stiffness (Young´s Modulus) and residual porosity of Mg-0.9Ca (X1) MIM-parts, measured by RFDA and Archimedes method

DISCUSSION & CONCLUSIONS: Generally, MIM enables the economic near net shape production of complex shaped parts in an industrial scale with high automation level. The processing benefits from the BE-route (blended elemental route) which enables an easy and flexible alloying of powdery base material (Mg) with elemental powders, MAP or even addition non-metal components. This offers a high degree of freedom in tailoring material properties, especially porosity. It is demonstrated that increased porosity, being beneficial for cell ingrowth and vascularisation, goes along with decrease of elastic modulus. With regard to future medical applications, the optimum degree of porosity and interconnectivity of Mg based biomaterials has to be evaluated.

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Submerged laser microcutting of Mg alloys with ns pulsed green laser for biodegradable stents

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INTRODUCTION: Laser microcutting is based on melt generation and expulsion (melt and blow) when continuous wave lasers are used. The use of pulsed lasers changes machining modality to ablation based cutting. Pulse duration becomes critically important and melt fraction can be reduced moving from long pulses (ms-µs) to short pulses (ns) and can be eliminated by the use of ultra-short pulses (ps-fs). Previous work determined that CW laser cutting allowed very high cutting speeds. On the other hand cutting with fs pulsed lasers allowed reduction of post processing and effective improvement of productivity in the whole production cycle of the biodegradable Mg alloy stents [1]. Another approach to laser microcutting is based on submerging the workpiece in a liquid and without the use of an assist gas [2]. Used with pulsed laser sources, the presence of liquid allows higher quality cutting by confining the ablation plasma, by increasing the mobility of molten material expelled from the cut kerf and by assisting machining through chemical action. In this work, submerged cutting with the use of 1 ns pulsed green fibre laser is studied. The effect of laser cutting parameters as well as liquid characteristics are investigated to achieve high quality cuts to reduce or eliminate any post processing stage in the manufacturing of biodegradable Mg stents.

METHODS: The laser source was a master oscillator power amplified (MOPA) fibre lasers operating with second harmonic wavelength 532 nm (YLPG-5 from IPG Photonics, Oxford, MA, USA), with the characteristics reported in Table 1. For workpiece positioning linear stages were used (ALS-130 from Aerotech, Pittsburgh, PA, USA) AZ31 sheets 0.25 mm thick were cut submerged in a liquid container (see Fig. 1). Deionized water, ethanol-water solution and machine lubricant oil were tested and compared to dry cutting conditions without the use of a process gas.

RESULTS: The preliminary results show that considerable differences in cut kerf morphology are present as a function of the submersion liquid (see Fig.2). The large kerf width obtained with the distilled water suggests that chemical reactions or mechanical interactions may take part in the process. High kerf quality was achievable with the ethanol-water solution.

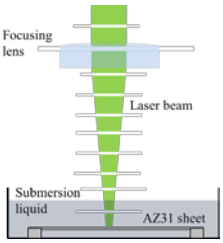

Fig. 1: Simplified scheme of submerged laser cutting of Mg alloy.

Table 1. General characteristics of the used laser source.

Wavelength	532 nm
Pulse duration	1 ns
Max. average power	6 W
Pulse repetition rate	20-300 kHz
Max. pulse energy	20 µJ
Beam diameter	22 µm

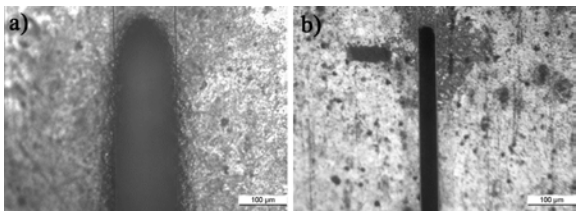

Fig. 2: Comparison between obtained kerf morphologies by submerged laser cutting in a) distilled water, b) ethanol-water solution.

DISCUSSION & CONCLUSIONS: The proposed method is promising for net shape stent manufacturing and has the potential to eliminate post chemical and electrochemical cleaning steps. The involved optical, mechanical and chemical phenomena are currently under investigation for a better comprehension of the process.

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ACKNOWLEDGEMENTS: The authors would like to thank IPG Italy for their support.

Mechanical properties of extruded antimicrobial Mg-Ag alloys

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INTRODUCTION: Biodegradable magnesium alloy has the advantage of avoiding the second surgery after fulfilling its function. However, infections pose a big challenge for surgical intervention[1], especially after the emergence of multi-drug resistant bacteria. To approach this problem, magnesium containing silver was proposed by researchers because of its relative good antibacterial property on the basis of biodegradable Mg-Ag binary alloy[2]. But how to improve the mechanical, as well as antibacterial properties is still an important focus. First results about mechanical properties of Mg-Ag alloys are presented in this study.

METHODS: Ingots of Mg6Ag and Mg8Ag were prepared by chilling mild steel mould into water (room temperature) during casting. To ensure all the second phase solutes into magnesium matrix, heat treatment (T4) is carried out at 430 ℃ for 16h. After preheat to 285 ℃ and 300 ℃ for Mg6Ag and Mg8Ag respectively those ingots were extruded into rods (Φ12) by hot extrusion with a high extrusion ratio of 108 and pre-programmed stable speed. The part where the rod has a full recrystallization was chosen to check micro-structure by optical microscope, measure hardness using hardness tester in HV5 mode and prepare standard tensile and compression samples. Five samples for each test were prepared by lathing.

RESULTS: The microstructure of as cast, heat-treated and extruded Mg6Ag and Mg8Ag is shown in Fig. 1. It can be seen that the second phase solutes into matrix after T4 treatment (b and e) compared to as cast alloys (a and d). After recrystallization during hot extrusion the grains were refined significantly.

The mechanical properties including hardness, yield stress, ultimate stress, elongation at break and E-Modulus are shown in Fig.2. The hardness had a trend of decrease after heat treatment; however, it increased after extrusion. Because of the higher silver content and finer grain, extruded Mg8Ag shows better yield tensile stress (YTS), ultimate tensile stress (UTS), yield compression stress (YCS) and ultimate compression stress (UCS). The elongation of Mg8Ag decreased compared with that of Mg6Ag, but Mg8Ag still shows high elongation which is about 20.9%.

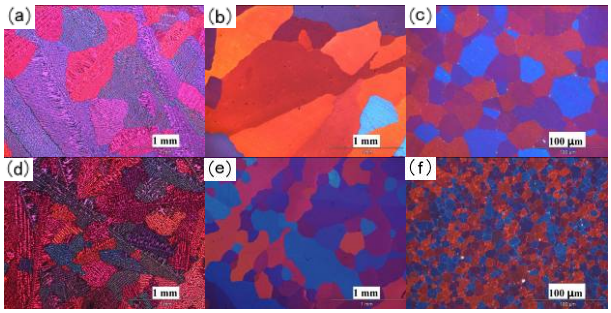

Fig. 1 Microstructure of Mg6Ag and Mg8Ag (a) as cast Mg6Ag (b) Mg6Ag after T4 (c) extruded Mg6Ag (d) as cast Mg8Ag (e) Mg8Ag after T4 (f) extruded Mg8Ag

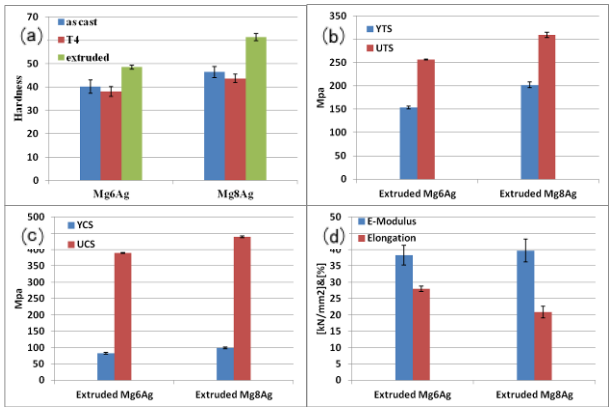

Fig. 2: Mechanical properties of Mg-Ag alloys

DISCUSSION & CONCLUSIONS: Higher silver content contributes to the fine grain of extruded alloys, improvement of hardness, yield and ultimate stress of tensile and compression. Both alloys have relative good elongation at break.

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ACKNOWLEDGEMENTS: The hot extrusion is done with the kind help of extrusion centre in TU Berlin.

Precise extrusion processing of Magnesium alloy small tube for biodegradable stent application

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INTRODUCTION: Mg has attracted attention as a biomaterial for biodegradable stent, because of biodegradability and low cytotoxicity [1]. However, it is difficult very much to fabricate a Mg stent tube precisely from low-plastic deformability due to its hexagonal closed packed structure. Generally the Mg stent tube is fabricated through a number of repeated drawing processes from an extruded large-diameter pipe, and the dimension accuracy of the extruded pipe very much affects that of the stent tube. Therefore the precise extrusion technique is essential to fabricate the stent tube with high dimension accuracy. Furthermore, developing the extrusion technique that doesn't use a porthole die conventionally is required to solve the problems of seams and inclusions for the pipe. The purpose of this study is to develop the precise extrusion technique without the porthole die and to fabricate the precise long-small Mg alloy tubes for reducing the number of processes.

METHODS: Four kinds of Mg alloy billets were prepared for small tube extrusion: AZ31, Mg-9%Al-1%Zn-2%Ca (AZX912 flame-resistant Mg alloy), Mg-2.5%Zn-6.9%Y (KUMADAI heat-resistant Mg alloy), and Mg alloy with high elongation and middle strength (BioMg). These billets were precisely extruded at 723 K into the tubes with different sizes: φ3.0 mm × L1000 mm (200 μm in thickness), φ3.2 mm × L700 mm (300 μm in thickness), and φ3.4 mm × L400mm (400 μm in thickness). The extruded tubes were cut, and the cross-sectional thickness measurement by an optical microscope and surface roughness measurement were made.

RESULTS: The extruded small tube has uniform wall thickness without any cracks and deformation (Figure 1). As shown in Table 1, the respective thickness errors were sufficiently low: ±3.69 % for AZ31, ±5.38 % for AZX912, ±1.77 % for BioMg, and ±4.57 % for KUMADAI alloy. Moreover, the surface roughness of both the surfaces shows low values of less than Ra 0.5 μm.

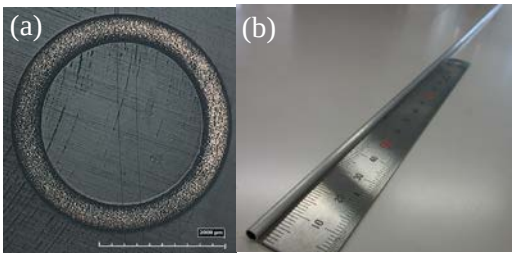

Fig. 1: Photograph of the precisely-extruded KUMADAI alloy tube with 400 μm in diameter: (a) cross section and (b) appearance.

Table 1. Thickness errors and surface roughness of the fabricated small tubes, and extruding load.

Mg alloy (thickness)	Thickness error /%	Ra /μm		Extruding load /kN
		Inner	Outer	
AZ31 (200 μm)	±3.69	0.3	0.5	49
AZX912 (200 μm)	±5.38	0.3	0.5	81
BioMg (300 μm)	±1.77	0.4	0.5	64
KUMADAI Mg (400 μm)	±4.57	0.3	0.5	93

DISCUSSION & CONCLUSIONS: We developed the precise extrusion technique for Mg alloy, and successfully fabricated the Mg alloy small tube with 1000 mm length in maximum and high dimension accuracy by precise extrusion. The higher strength Mg alloy has the tendency to decrease the dimension accuracy of the extruded tube with increasing the extruding load. This result indicates the control of extruding load contributes to the improvement in the dimension accuracy of the extruded tube. Moreover, it is found that the precise extrusion of Mg alloy is achieved even in any alloy compositions by optimizing the processing parameters.

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Evaluation of Magnesium alloys for Tracheal Stent Application

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INTRODUCTION: Tracheal stenting is used for successful management of adult airway obstructions; including tracheal stenosis.[1] The permanent nature of non-degradable tracheal stents makes them a last resort treatment option specially for pediatric patients. Complications related to stent removal and restenosis could be avoided with a degradable tracheal stent placement. Magnesium alloys have shown promise as degradable materials for orthopedic and cardiovascular applications.[2,3] In our previous study, AZ31 alloy exhibit the best overall degradation and biocompatibility in rat trachea model. In this study, we fabricated a novel ERC-P-06 alloy based on First Principles calculations and explored the potential of this alloy for use as degradable tracheal stents.

METHODS: Pure elemental metals used as initial materials were melted at 720°C. The ingots were T4 heat treated and extruded with an extrusion speed of 1 mm/s. To study the *in vitro* degradation behaviour of ERC-P-06 alloy, samples were immersed in Hanks' solution for 1, 2 and 3 weeks according to the ASTM G31 standard. Corrosion rate was calculated based on weight loss and the corrosion layer was assessed by SEM/EDX. Mechanical properties were assessed based on ASTM E8 standard. For primary cytotoxicity evaluation, MTT test was conducted on BEAS-2B cell line (human bronchial epithelial cells).

RESULTS: The gran size (**Fig.1**) was greatly refined after extrusion. In the transverse direction, typical undefined grains characteristic of extrusion are visible due to severe plastic deformation. The grains were elongated along the extrusion direction. The corrosion resistance of ERC-P-06 alloy was significantly improved after extrusion (**Fig.2**). Compared to AZ31, even though the corrosion rate of extruded ERC-P-06 alloy is slightly higher, the overall corrosion appears to be more uniform. Due to grain refinement, the strength and ductility were significantly improved after hot extrusion. BEAS-2B cell viability results show good cytocompatibility of ERC-P-06 alloy.

DISCUSSION & CONCLUSIONS: ERC-P-06 alloy exhibits potential for use as degradable intra-luminal stents for tracheal obstruction. Extruded alloy shows less pitting, higher corrosion resistance and optimized mechanical properties. Future plan would be to conduct *in vivo* studies to evaluate ERC-P-06 alloy in rabbit tracheal model.

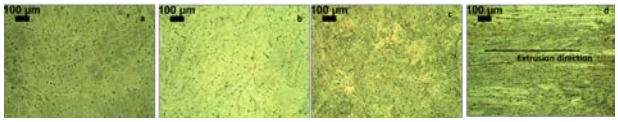

Fig. 1: Microstructure of ERC-P-06 alloy (a) as cast, (b) T4 treated, (c) as extruded, transverse section, and (d) as extruded, longitudinal direction.

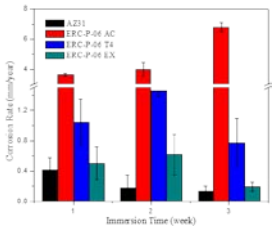

Fig. 2: In vitro degradation behaviour of ERC-P-06 alloy in Hank's solution.

Table 1. Mechanical properties of ERP-P-06 alloy.

	YS (MPa)	UTS (MPa)	EL (%)
As-Cast	90.8±7.4	129±13	3.6±0.4
T4	84.2±9.3	134±10	3.5±1.0
Extruded	233.5±4.2	283±2	8.6±2.1

REFERENCES: [1] Y. Saito, H. Imamura (2005) *Surgery today* **35:**265-70. [2] J. Walker, S. Shadanbaz *et al* (2014) *J Biomed Mater Res B* 3 M. Haude, R. Erbel (2013) *Lancet* **381**:836-44.

ACKNOWLEDGEMENTS: Support of NSF funded Engineering Research Center-Revolutionizing Metallic Biomaterials (RMB) via grant-EEC-0812348 is gratefully acknowledged.

Biodegradable Mg alloy with modified nanoscale surface for stent applications

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INTRODUCTION: Magnesium (Mg) alloys are emerging as a revolutionary biomaterial for cardiovascular stent application based on the attracting biodegradability of the substrates that are gradually broken down in human body [1]. However, rapid degradation of Mg alloys under physiological conditions has shown to be clinically ineffective due to early disintegration of the load-bearing implants before the tissue has sufficiently healed [2]. Here we report a novel Mg alloy Mg-Nd-Zn-Zr (JDBM) modified with 0.1 M potassium fluoride (KF) and also develop a better understanding of the mechanism for the surface modification leading to an improved biological response to the Mg alloy.

METHODS: The JDBM specimen (Ø12×5 mm) was chemically treated in 0.1M KF solution for 48 h at room temperature to allow the conversion MgF_2 film formation. Immersion test and electrochemical measurement were performed in artificial plasma at 37 °C and buffered at pH =7.4. Human umbilical vein endothelial cells (HUVECs) and THP-1 derived macrophages were used to evaluate the biological response to the Mg alloy materials by direct cell assay.

RESULTS: The surface physico-chemical properties of the implanted material have significant effects upon production of local microenvironments which profoundly influence cellular behavior in terms of adhesion strength, spreading, intracellular signaling and differentiation potential [3]. The consequences of cell adhesion and spreading differ markedly by the surface with different physico-chemical properties of the two samples (Fig. 1a-d). A few cells are observed on the naked JDBM surface after 1 day culture, while the adhesive cell density increases distinctly on the MgF_2 nanoscale surface. After 2 days incubation, we observed elongated cell configuration with pseudopodium well adhered on the JDBM alloy with MgF_2 film, and the cell morphology is similar as those cultured in normal tissue culture plate, indicating the nanoscale MgF_2 surface support a favorable culture environment for the growth of primary endothelial cells.

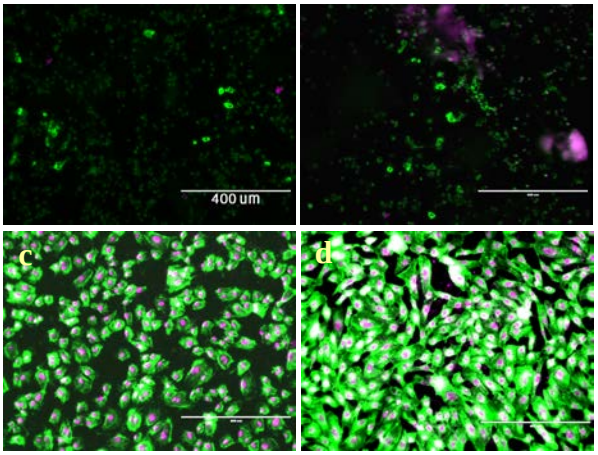

Fig. 1: Direct HUVECs adhesion on Mg alloy substrates. Cell growth on naked JDBM for 24 h (a), 48 h (b) and on nanoscale MgF_2 surface for 24 h (c), 48 h (d).

DISCUSSION & CONCLUSIONS: We have demonstrated that the nanoscale MgF_2 film favors HUVECs deposition and potentially aids in endothelialization of the implant into the denuded artery. In general, medical devices such as scaffold and prosthesis have a low affinity for cell adhesion and function. Due to rapid kinetics, biomaterials and medical devices exposed to blood immediately acquire a layer of plasma and extracellular matrix (ECM) proteins prior to interacting with host cells. The surface properties of biomaterials such as surface structure, wettability and surface charge might be critical for the distribution of proteins and their native conformation on an implant surface. To enable the anastomosis of medical device to the surrounding host tissue, surface modifications are commonly used to improve the assembly and alignment of ECM and cell affinity. The KF treatment offers an alternative strategy to meet this goal and the nanoscale MgF_2 film shows great potential for Mg-based cardiovascular stent applications.

REFERENCES: [1] M. Haude, R. Erbel, P. Erne , et al (2013) *The Lancet* **381**: 836-44. [2] G. Song, A. Atrens (2003) *Adv Eng Mater*. **5**: 837-58. [3] C. J. Shen, S. Raghavan, Zh. Xu, et al (2011), *Experimental cell research* **317**: 1860-71.

The effect of hot rolling on the microstructure and degradation behaviour of a Mg-Ca-Sr alloy

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INTRODUCTION: Understanding degradation behaviour is critical for Mg biodegradable implant applications since rapid hydrogen gas evolution can cause adverse tissue reactions while compromising the mechanical support required by the implant during healing. Recently, a Mg-Ca-Sr alloy was developed [1], which shows minimal toxicity to bone cells and a reduced degradation rate in its as-cast condition compared to comparable binary alloys [2-3]. It has been shown that microstructural refinement affects degradation, with small grain size and more homogenous structure with a finer distribution of precipitates generally reducing the degradation rate [2, 4]. This study investigates the effect of hot working on microstructural evolution and its associated in-vitro degradation response.

METHODS: A Mg-1.3Ca-0.8Sr (wt.%) alloy was cast[1] and then homogenized at 450°C for 24h under flowing Ar, followed by furnace cooling. The composition was confirmed by inductively coupled plasma atomic emission spectroscopy. The cast was cut and polished into 45x10x5mm pieces which were rolled at 400°C by preheating in a furnace and reheating for 5min between each pass, resulting in a total thickness reduction after all passes of 29.5%. Grain size was measured by ASTM E112. Degradation behaviour of homogenized and rolled specimens was determined by immersion testing in static Hanks' balanced salt solution at 37°C, by collecting and measuring the hydrogen gas evolved [5]. Degradation tests were performed in triplicate in air and at atmospheric pressure.

RESULTS: The homogenized and rolled alloys appeared to have uniform and equiaxed grains with an average size of 547.0µm and 47.3µm, respectively. Precipitates were located in the interdendritic region in the homogenized alloy and primarily located along grain boundaries after rolling. Figure 1 shows the microstructure and hydrogen evolution of the alloy in its homogenized and rolled condition. Hydrogen evolution rates of the alloy in the as-cast condition and comparable binary alloys are shown for comparison. As can be seen, the rolled specimens degrade an order of magnitude slower than in the homogenized condition, which is similar to the as-cast rate. All specimens appeared to degrade uniformly, with only minor corrosion product built-up on the surface. No significant change in pH of the Hanks' solution was observed during the degradation.

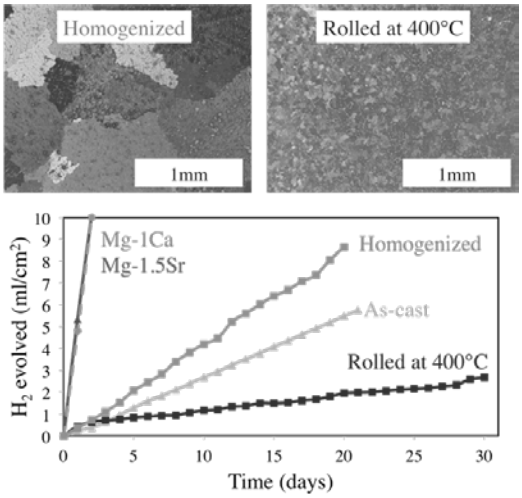

Fig. 1: Microstructures and hydrogen evolution rates of homogenized and rolled specimen. Degradation profiles of the as-cast condition [1] as well as cast binary alloys [2,3] are shown for comparison.

DISCUSSION & CONCLUSIONS: In this study it was shown that hot rolling of Mg-1.3Ca-0.8Sr alloy refines the microstructure which affects the in-vitro degradation behaviour of the alloy. Equiaxed small grains in rolled samples suggest dynamic recrystallization taking place during rolling. The overall refined microstructure of rolled samples resulted in slower degradation, with rates an order of magnitude lower than the as-cast[1] and homogenized equivalents. It was also observed that the homogenized specimens degraded faster than as-cast ones, which can be attributed to grain growth during homogenization.

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Synthesis and Characterization of Biodegradable Magnesium-Yttrium based Alloys for Musculoskeletal Load-bearing Medical Implants

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INTRODUCTION: Permanent inert metallic biomaterials currently used for orthopedic and craniofacial devices are known to cause complications which have led to increased interest in biodegradable metals. Degradable Mg alloys have emerged as promising alternatives due to similar mechanical properties to natural bone and good biocompatibility. However, rapid corrosion of Mg may lead to premature mechanical failure resulting in possible adverse reactions. In order to improve the mechanical properties and corrosion resistance, novel magnesium based alloys were developed following theoretical First Principles calculations and further improved using extrusion.

METHODS: Pure magnesium and other high purity alloying elementals were melted in an electrical resistance furnace between 720-750 °C. After stirring, the molten metal was poured into a mild steel mold. The as-cast alloys (termed ERC-P-##) were further solution treated and hot extruded. Alloy microstructure was observed under optical microscopy. Tensile mechanical testing was conducted following ASTAM E-08 at an extension rate of 1.3 mm/min. MC3T3 pre-osteoblast cells were cultured directly on the alloys at a density of 4×10^4 cells/ml. Viability of seeded cells was evaluated after 1 and 3 days using the live/dead assay. Cells attached on the samples were imaged using fluorescence microscopy.

RESULTS: Extrusion resulted in significant grain refinement as observed by optical microscopy. Mechanically properties of extruded alloys were also superior to their as-cast forms, with strengths greater than commercially obtained extruded pure Mg and AZ31. The direct live/dead assay (**Fig. 1**) showed higher density of attached live cells on extruded alloys after 3 days of culture compared to other conditions. Higher live cell attachment was also observed on ERC-P-12 alloys compared to ERC-P-11 alloys after 3 days of culture (**Fig. 2**).

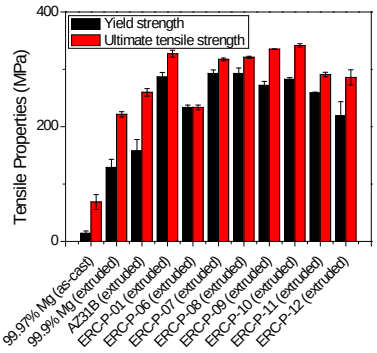

Fig. 1: Tensile mechanical properties (yield strength and ultimate tensile strength) for extruded alloys compared to pure Mg and AZ31.

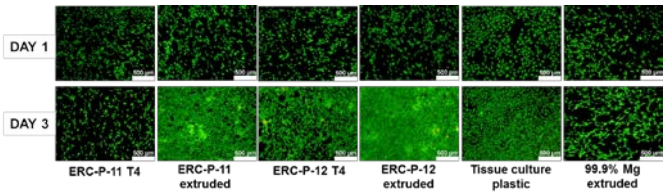

Fig.2: Fluorescence microscopy images of MC3T3 after performing the live/dead assay following 1 and 3 days of culture on the alloys.

DISCUSSION & CONCLUSIONS: Extrusion resulted in significant improvement in mechanical properties and cell viability of the Mg alloys studied in this work. Grain refinement led to strengthening in agreement with the Hall-Petch relation wherein grain boundaries act as pinning sites impeding dislocation movement. Higher live cell attachment on ERC-P-12 alloys compared to ERC-P-11 alloys may have been observed due to P-12 consisting of higher rare-earth content leading to a more stable passive layer.

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Mechanical and corrosion properties of UFG Mg alloys

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INTRODUCTION: Magnesium alloys have been proposed in medical science as a novel class of highly bioactive materials. That is, these materials are supposed to temporarily aid the healing process of a diseased tissue and then progressively disappear after a certain length of functional use. Previous studies proved that grain refinement of magnesium alloys is the most efficient way to improve both mechanical and corrosion properties due to Hall-Petch strengthening and homogeneous distribution of precipitates, respectively. In the present study, the effect of UFG structure obtained by Equal Channel Angular Pressing (ECAP) on mechanical properties, texture and corrosion behaviour of ZK60 alloy are reported.

METHODS: A commercial ZK60 wrought alloy was used in an ECAP process according to a multi-step strategy to achieve at first refining of the structure and then reaching the UFG grain size range by still reducing the processing temperature. Grain orientation maps of the alloy in the as-received and UFG conditions was obtained by using Electron Backscattered Diffraction (EBSD) interfaced with a field-emission gun scanning electron microscopy (FEG-SEM). Mechanical properties were characterized by tensile and compression tests. In order to obtain a thorough understanding of the type of corrosion mechanism, microstructural characterization of the corroded samples was carried out by FEG-SEM studies.

RESULTS: Fig. 1 shows EBSD maps of the as-received and ECAP processed alloy. The as-received sample featured a heterogeneous microstructure along with bands of secondary phase particles distributed along the extrusion direction. Eventually, a uniform equiaxed UFG microstructure was achieved through ECAP process. A collection of tensile and compression results referred to the as-received and UFG alloy are listed in table 1. The corrosion sub-layer of as-received sample can be observed in Fig. 2a. It is seen that due to the corrosion attack, grains are appearing in regions with signs of preferential direction of corrosion advancement. It is supposed that the large holes are formed in the regions where a primary accumulation of second phase particles existed. In UFG samples, all particles are broken into fine sizes, dispersed homogeneously in the matrix (Fig. 2b).

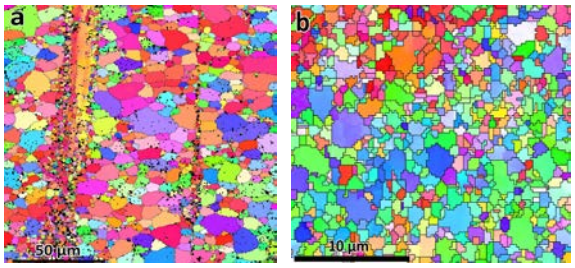

Fig. 1: EBSD inverse pole figure of ZK60: As-recieved (a) and ECAP processed (b).

Table 1. mechanical properties of the alloys before and after ECAP.

Sample condition	TYS (MPa)	UTS (MPa)	CYS (MPa)	Fracture el. (%)
As-received	292	343	165	16
UFG	267	296	253	30

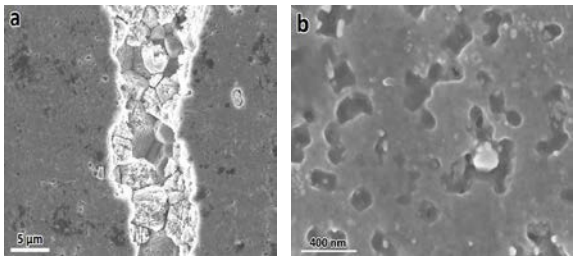

Fig. 2: SEM micrographs of (a) as-received and (b) UFG alloy immersed in PBS after 4h.

DISCUSSION & CONCLUSIONS: Mechanical analyses showed that a combination of grain refinement and texture development led to an improvement of both strength and ductility in UFG alloy. Morphological studies of corroded surfaces demonstrated that remarkable localized corrosion was observed in the as-received sample around the accumulation areas of the large second-phases, while in UFG sample, a shift of corrosion regime to a more uniform corrosion mode was observed mainly due to the second phase refinement and redistribution.

In vitro degradation of pure magnesium and WE43 alloy in human bile

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INTRODUCTION: There are limitations of currently used stents for the treatment of biliary strictures. Permanent metal stent (often made of titanium) may induce sludge accumulation and epithelial hyperplasia, while polymeric stent (made of PE, PVC, PU or PTFE) include lack radial force. Biodegradable biliary stents made of magnesium and its alloys may address the problems. In this study, we evaluated the *in vitro* degradation behavior of pure Mg and WE43 in human bile.

METHODS: The bile was collected from patients underwent T-tube drainage in People's Hospital, Peking University with permission. Disk samples of as-cast pure Mg and as-extruded WE43 alloy with dimensions of 10×10×2 mm^3 were immersed in 50 ml bile and the temperature was kept at 37°C by water bath. The hydrogen evolution during immersed was monitored. The weight loss of the samples after immersion was measured. The inductively coupled plasma atomic emission spectrometry (ICP-AES) was employed to measure the concentrations of magnesium ions in the bile. Changes on the sample surface of samples were characterized by environmental scanning electron microscopy (ESEM) and x-ray diffraction (XRD).

RESULTS: The hydrogen evolution rate declined with immersion time due to the formation of corrosion product layer. After 48h immersion, the hydrogen evolution rate of both pure Mg and WE43 alloy decrease to below 1ml/cm^2/d. Surface morphologies of pure Mg and WE43 alloy after 60d immersion in human bile are shown in Fig.1. The surfaces were still smooth with no obvious corrosion pit can be observed. XRD analysis revealed that the corrosion product of pure Mg and WE43 alloy in human bile was mainly $Mg(H_2PO_4)_2$. Both results of ion release and weight loss indicated WE43 alloy exhibited better corrosion resistance than pure Mg. The weight loss of pure Mg and WE43 alloy after 60d immersion in human bile were 1.17% and 0.12%, respectively.

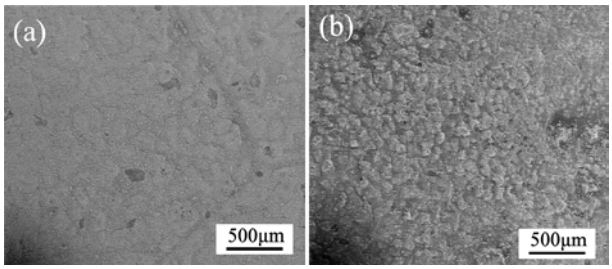

Fig. 1: Surface morphologies of (a) pure Mg and (b) WE43 after 60d immersion in human bile.

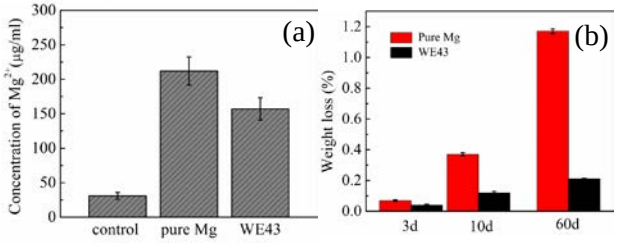

Fig. 2: (a) The magnesium concentration of the bile after 60d immersion, (b) weight loss of pure Mg and WE43 alloy samples after different immersion duration.

DISCUSSION & CONCLUSIONS: Chen et al. [1] implanted Mg–6Zn stents in rabbits and found only 9 % of the original weight remained after 3 weeks, which cannot meet the clinical requirement. In the present study, both pure Mg and WE43 exhibited much lower degradation rate. WE43 alloy, which is outperforming pure Mg, is promising for biodegradable biliary stents material. Further *in vivo* evaluation should be done to verify the safety and efficiency.

REFERENCE: [1] Y. Chen, J. Yan, C. Zhao, et al (2014) *J Mater Sci: Mater Med* **25:**471–10.

ACKNOWLEDGEMENTS: This work was supported by the National Basic Research Program of China (973 Program) (Grant No. 2012CB619102 and 2012CB619100) and National Science Fund for Distinguished Young Scholars (Grant No. 51225101).

Magnesium corrosion under physiological conditions

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INTRODUCTION: Magnesium and its alloys as degradable metals have considerable potential for orthopaedic applications. During the degradation process the interface between material and tissue is continuously changing. Moreover, too fast or uncontrolled corrosion is detrimental for the outcome in vivo. Therefore in vitro setups utilizing physiological conditions are promising for the material / degradation analysis prior to animal experiments. However, not all mechanisms are fully understood yet. The aim of this study was to elucidate the influence of inorganic salts, which contribute to the blood buffering capacity, on the corrosion process.

METHODS: Extruded pure magnesium samples (d=10 mm, t=1.5 mm) were immersed under cell culture conditions for 3 and 10 days. Hank's balanced salt solution without calcium and magnesium (HBSS) plus 10% of fetal bovine serum (FBS) was used as basic corrosion medium. Additionally, different inorganic salts where added with respect to concentration in Dulbecco's modified Eagle's medium (DMEM) and human plasma to form 12 different corrosion mediums. During the immersion period the immersion solution was changed every three days. Both pH and osmolality for the extract were measured at each medium change. At the end of immersion time the corrosion rate by mass loss was calculated. The concentrations of magnesium, calcium and phosphorous in the corrosion medium after the 3 days immersion were measured by inductive coupled plasma mass spectroscopy (ICP-MS). The corrosion layer was analysed by electron-induced X-ray emission microanalysis spectroscopy and Fourier transform infrared reflection micro-spectroscopy (FTIR) to map the chemical elements distribution and their possible chemical compositions.

RESULTS: ICP-MS results on 3 days immersion extracts revealed a general trend of decrement in phosphorous and calcium concentration for all conditions, conversely these elements were mapped in the corrosion layer in proportional way to their initial concentration in the immersion media. These calcium-phosphorous depositions had an influence on degradation behaviour by decreasing corrosion rate.

Adding carbonate containing salt increased the corrosion rate as well magnesium concentration in extract, but on contrary its addition with calcium containing salts enhanced the effect of calcium in decreasing the corrosion rate.

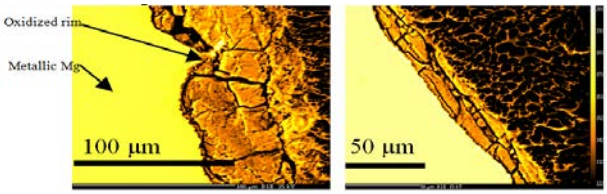

Fig. 1: Back scattered image for corrosion layer after 10 days immersion in two different mediums, the basic solution (BS) for both is Hank's Balanced salt solution plus 10 %FBS; left: BS+ 3350 mg/L $NaHCO_3$; right: BS+ 264 mg/L $CaCl_2$ + 200 mg/L $MgSO_4$ + 3350 mg/L $NaHCO_3$

DISCUSSION & CONCLUSIONS: The results highlight the importance of salts containing calcium and bicarbonate on the degradation process: while calcium tends to form kind of calcium-phosphates homogenous protecting layer, the bicarbonate enhances the corrosion process by forming heterogeneous layer basically consisting of magnesium carbonate as a corrosion product. $MgSO_4$ had no significant effect on the corrosion rate, although it influenced the corrosion layer morphology.

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Influence of deformation on the corrosion and cytocompatibility of biodegradable Mg alloys

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INTRODUCTION: Mg alloys have been widely accepted as promising biomaterials for biodegradable implants. Many Mg alloys such as WE43, MgCa0.8, MgYREZr, ZEK 100, LAE442 etc., have been fabricated into screws or stents for animal models and even for clinical trials [1]. However, previous *in vivo* tests of these Mg alloys have shown exceedingly high degradation rates and toxicity concern of some of the alloying elements associated with Mg alloys. As a result of these disadvantages, Mg alloys are limited in their use as effective bio-metals. Apart from alloying elements, deformation is the most important factor for materials scientists to tailor to increase the performance of Mg alloys.

DEFORMATION: Deformation is an important process in order to make tubes, rods, wires, and other related products. It has been well-known that deformation can refine grain size and improve the mechanical properties of metals according to the classic Hall-petch relationship. Since there is a high density of grain boundaries and dislocations, as well as the re-distribution of particles induced by deformation, it is reasonable to predict that the corrosion behaviour and the cytocompatibility are altered as well. However, the influence of deformation on the corrosion behaviour and cytocompatibility of Mg alloys is still not clear.

CORROSION: Most studies showed that deformation, especially severe plastic deformation (SPD), has positive influence on the corrosion of Fe alloys, Ti alloys, Al alloys, and Mg alloys. The reason was believed to be because of the ability of high grain boundary density surfaces to passivate more readily or to the physical breakdown of the second phases/particles [2]. Ralston et al. [2] reported on the relationship between grain size and corrosion rate of metals and proposed that a similar Hall-petch relationship could be considered between corrosion rate and grain size when an oxide/passivity existed. Chen et al. [3] studied the influence of extrusion ratio on the mechanical properties, corrosion resistance, and cytocompatibility of pure Mg. The results showed that extrusion improved the corrosion resistance greatly. Earlier reports revealed that deformation of AZ31, Mg-Nd-Zn-Zr, Mg-Ca, ZK60 etc., improved corrosion resistance compared to the same alloys in the as-cast condition [1].

CYTOCOMPATIBILITY: The selection of elements for alloying should avoid toxic elements. However, all elements have their own tolerance limits in the human body. Therefore, it is very important to control the corrosion rate of the developed alloys in the application environment. Deformation can significantly improve the corrosion resistance which can lead to better cytocompatiblity. Bindu et al. [4] reported that ultrafine grained (UFG) pure titanium induced by severe plastic deformation had better biocompatibility over coarse grained titanium. The improved biocompatibility of UFG-Ti was attributed to the presence of surface discontinuities, surface energy, higher wettability, surface stress, and stable oxidized films. There are few similar studies on Mg alloys and further studies on Mg alloys are needed.

CONCLUSIONS: Deformation is very significant to tailor microstructure, mechanical properties, corrosion behaviour, and cytocompatibility of Mg alloys in order to fabricate properties controllable Mg alloys for biodegradable implants.

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Inflammatory Response to Magnesium Based Biodegradable Implant Materials

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INTRODUCTION: The biodegradability and the mechanical properties of magnesium alloys make them a suitable material for orthopaedic implant applications. Several papers are studying the interaction of bone cells with these materials [1, 2]. Still during implantation a variety of cells is interacting. Among them are cells of the immune system which are responsible for the inflammatory reaction of the organisms. Macrophages play a central role in the inflammatory process and their interaction with the degrading material is consequently the target of this study.

METHODS: In order to evaluate the influence of ion release, pH changes and hydrogen evolution produced by degrading magnesium alloys on the inflammatory process, undifferentiated (monocytes) and PMA (phorbol myristate acetate) stimulated (macrophages) U937 cells were cultured with three different magnesium alloys: Mg4Y3RE, Mg10Gd and Mg2Ag. Two different culture methods were employed: the cells were either exposed to an extract of the degrading material or grown directly on the material. For the extract assay the three materials were incubated under cell culture conditions for 72hin cell culture media. During this time the materials degraded and extracts with defined Mg concentrations (measured by Xylidyl Blue Assay) were obtained. Then the cells (5 x 10^5 cells/mL) were subsequently cultivated with these extracts (diluted 1 to 10 to decrease the osmolality) for 1 and 10 days. For the direct assay the cells (5 x 10^5 cells/mL) were cultured on the surface of discs (1mm x 10mm) for 1 and 6 days. Cells without material contact were used as control. The inflammatory activity of the cells was assessed by a Human Cytokine Array Panel(Fig. 1) which is a qualitative method for the simultaneous analysis of 36 cytokines. In addition standard and multiplex ELISA (Qplex) measurements were performed to detect inflammatory cytokines in the cell culture supernatant. The expression of specific genes such as CD36 (Cluster of Differentiation 36 or thrombospondin receptor), TLR4 (Toll-Like Receptor 4), ICAM1 (Intercellular Adhesion Molecule 1), IL-1β (Interleukin 1β) and OPN (Osteopontin) were obtained by real time Polymerase Chain Reaction (RT-PCR). Every sample was tested in triplicates.

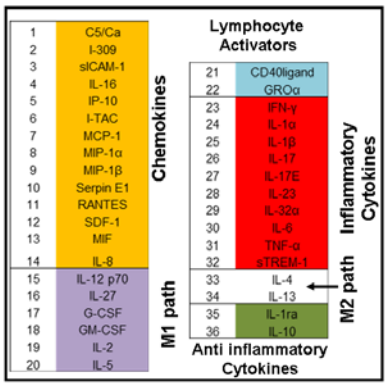

Fig. 1: Overview of analysed cytokines.

RESULTS AND DISCUSSION: Undifferentiated U937 (monocytes): In presence of extracts, no production of inflammatory cytokines was detected on protein and gene level. For PMA stimulated U937 (macrophages): The analyses revealed that when cells are growing directly on the Mg alloys, the material can stimulate differentiated U937 cells (macrophages) to release chemotactic factors such as IL- 8 (Interleukin-8) and MCP1 (Monocyte Chemotactic Protein-1). Down regulation of inflammatory genes is observed when the cells are cultivated with extracts.

CONCLUSION: Our results indicate that at least for the three Mg alloys tested two mechanisms depending on the maturation of cells (monocytes / macrophages) play a role: (1) a recruitment of monocytes towards the damaged tissue is possible and (2) when differentiated into macrophages the material can be involved on the down regulation of the inflammatory reaction.

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Suitability of novel PLA/Magnesium composites for biodegradable implants

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INTRODUCTION: Implants that can be metabolized by the human body have appeared as one of the most attractive and promising solutions to overcome limitations and improve the features of current implantable devices. Biodegradable polymers and magnesium (Mg) alloys have played an important role writing the history of resorbable implants [1,2]. This paper presents the processing by extrusion/compression moulding, mechanical characterization, thermal characterization and *in vitro* biocompatibility of a novel generation of resorbable materials based on a polymeric matrix reinforced with metallic Mg particles.

METHODS: Mg particles (<50 μm) of irregular shape were used to reinforce a polymeric matrix of poly-D-L-lactic acid (PLDA) with 1% and 10% of Mg weight content. Composites were fabricated by extrusion obtaining filaments that were pelletized in a further step. Pellets were moulded by compression into discs for cell culture and cylinders for mechanical tests. Mechanical properties were assessed by compression tests at different strain rates to determine the strain rate dependence of the material. Thermal properties were studied by DSC and TGA. *In vitro* biocompatibility of the samples was analysed using human mesenchymal stem cells (MSCs). Cell viability was evaluated using the non-invasive AlamarBlue assay and morphology of cells stained for actin filaments and nuclei was visualized by CLSM.

RESULTS: Materials reinforced with Mg particles exhibit higher compressive strength and Young´s modulus than the neat polymer. Young´s modulus of the samples is higher with increasing strain rate. PLA/Mg mechanical properties meet the values of cortical bone. Thermal behaviour of composites is stable in the temperature range Tamb-300ºC. Beyond 300ºC, Mg accelerates PLDA thermal degradation. Results from *in vitro* studies show that Mg reinforcement improves viability of MSCs cultured on the materials (Fig. 1).

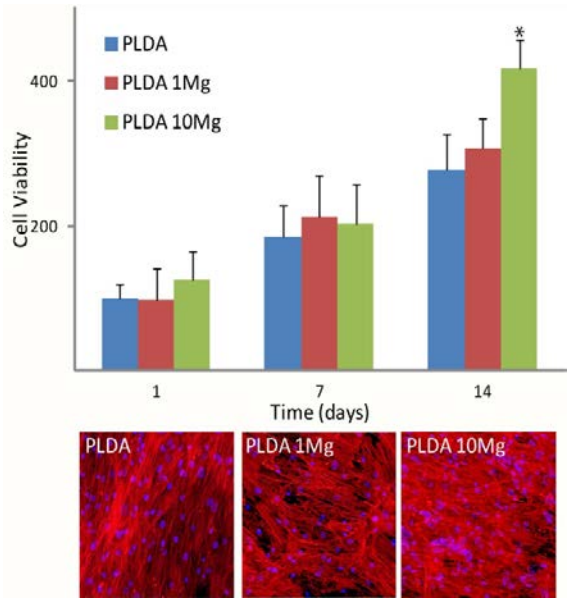

Fig. 1: Cell viability and morphology on PLDA/Mg composite.

DISCUSSION & CONCLUSIONS: When Mg is combined with a polymeric matrix a synergistic effect takes place and the disadvantages of one material are mitigated by the advantages of the other. On the one hand PLDA controls and slows down Mg particles corrosion; on the other hand Mg improves the mechanical properties of the polymer and plays a beneficial role on its biocompatibility. PLA/Magnesium composites are novel materials that can be suitable for resorbable implants.

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Metallic magnesium degradation products inhibit human breast cancer bone metastasis via multiple signaling pathways

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INTRODUCTION: Bone metastasis is a common and serious complication of advanced cancers, such as breast cancer, prostate cancer, and multiple myeloma. The life expectancy of patients with malignant tumors and the incidence of osseous metastases have increased over several decades. Surgical treatment of skeletal metastases of the extremities represents the most frequent surgery performed in orthopedic oncology. At present, surgeons recommend intralesional resection of the metastasis and stabilization with orthopedic implants as the treatment of choice for pathologic fractures of the humeral or femoral diaphysis and metaphysis. Thus, the development of materials that inhibit tumor invasion and bone lesions would be a promising strategy for preventing tumor recurrence. Magnesium (Mg) has been used in orthopedic implants for a long time. Because of its superior properties for reducing breast cancer bone metastasis and induced osteolysis, we examined the effects of metallic magnesium degradation products (MDP) on human breast cancer cells.

METHODS: We mimicked the *in vivo* process of Mg degradation, to obtain MDP, by immersing pure Mg in the culture medium. *In vitro*, we examined the inhibitory activity of MDP on human breast cancer cell (MDA-MB-231) viability, apoptosis, invasion, migration, and MDP-induced osteoclast formation and function using cell count kit-8, flow cytometry, and transwell and co-culture assays. *In vivo*, we used a mouse xenotransplant model with luciferase-labeled MDA-MB-231 cells to determine the effects of MDP on breast cancer bone metastasis and cancer cell-induced osteolysis via MDP-injection into tumor sites.

RESULTS: *In vitro* MDP inhibited MDA-MB-231 cell proliferation, pro-apoptosis, suppressed invasion and migration activity, and attenuated MDA-MB-231-induced osteoclastogenesis and bone resorption. *In vivo*, MDP suppressed bone tumor growth and tumor-induced osteolysis. In a molecular mechanism analysis, we noted that MDP inhibited breast cancer bone metastasis and cancer cell-induced osteolysis via multiple ways. (1) *In vitro*, MDP inhibited the mitogen-activated protein kinases (extracellular signal-regulated kinase and p38)-dependent matrix metalloproteinase-9 signaling pathway in MDA-MB-231 cells. (2) MDP down-regulated serum tumor necrosis factor-α, interleukin (IL)-6, and IL-1b levels, *in vivo*, thus alleviating inflammation-triggered, pro-tumor effects. (3) MDP inhibited osteoclast formation and function in co-culture systems, possibly suppressing tumor burden-induced bone lesions, *in vivo*, by directly targeting osteoclasts.

DISCUSSION & CONCLUSIONS: Collectively, these results suggest that metallic MDPs had anti-tumor activity and that magnesium or magnesium-alloys might be developed as effective anti-tumor material for preventing breast cancer bone metastasis or recurrence.

*Contributed equally; #Co-corresponding authors.

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Effects of extracellular magnesium extracts on the proliferation and differentiation of human osteoblast-osteoclast co-cultures

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INTRODUCTION: Up to now, *in vitro* biocompatible assessment for biomedical magnesium-based alloys have been predominantly focused on the response to osteoblasts and their precursors of murine or human origin by analyzing their biocompatibility, osteoconductivity or osteoinductivity [1-3]. Recently we reported the effect of magnesium on human osteoclast monocultures. However, *in vivo* osteoblasts and osteoclasts are usually both present on the bone surface and interact with each other. Therefore co-cultures containing bone-forming osteoblasts and bone-resorbing osteoclasts for *in vitro* biomaterial testing are one step closer to natural conditions.

METHODS: In the present study, SCP-1 stem cells were co-cultivated with peripheral blood mononucleated cells (PBMC) for 28 days (without the external addition of osteoclastogenesis promoting factors receptor activator of nuclear factor kappa-B ligand (RANKL) and macrophage colony-stimulating factor (M-CSF)) in order to differentiate into osteoblasts and osteoclasts, respectively. Concomitantly, the cultures were exposed to seven variable magnesium extracts dilutions (0, 30x, 10x, 5x, 3x, 2x and 1x with Mg^{2+} concentrations at 0, 0.89, 2.67, 5.34, 8.90, 13.35 and 26.67 mM, respectively). Proliferation and differentiation of osteoblasts as well as osteoclasts were evaluated based on light microscopy, lactate dehydrogenase (LDH) cytotoxicity assay, alkaline phosphatase (ALP) activity, tartrate-resistant acid phosphatase (TRAP) activity, and RANKL, M-CSF, osteocalcin, osteoclast associated receptor (OSCAR) Elisa assays.

RESULTS: Phenotype characterization performed by light microscopy at day 28 documented that while 2x magnesium extracts dilution is extremely toxic to osteoclast solo-culture, monocytes in co-cultivation with SCP-1 exhibits a higher tolerance to magnesium extracts (Fig. 1). The results of ALP activity as well as TRAP activity revealed significantly enhanced formation of osteoblasts and decreased osteoclastogenesis in the cultures with lower dilutions of magnesium extracts (3x, 2x and 1x).

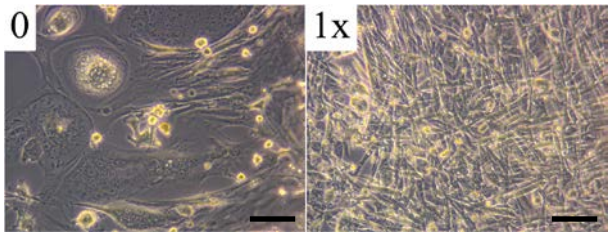

Fig. 1: Light microscopic images of co-cultures at day 28 (scale bar = 100 µm). Left, without magnesium extracts; Right, with 1x magnesium extracts.

DISCUSSION & CONCLUSIONS: Co-cultures containing both bone-forming osteoblasts and bone-resorbing osteoclasts should be preferentially performed for *in vitro* biocompatibility assessment of magnesium-based implants as they more closely mimic the *in vivo* environment. While a contribution of osteoblasts to increased osteoinductivity as well as enhanced bone formation has been demonstrated, the impact of potentially decreased osteoclastic resorption around the magnesium-based implants should be also taken into account. These results are consistent with the *in vivo* investigations conducted by Janning et al. [4] in which an enhanced osteoblast activity/bone formation and a temporal decrease in osteoclast number in peri-implant bone remodeling were found.

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Behavior of bone cells due to contact to magnesium implant material

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INTRODUCTION: Magnesium-based implants exhibit several advantages like biodegradability and enhancement of *in vivo* bone formation [1]. Nonetheless, the degradation may induce cell type specific changes in metabolism which still stay unclear. To examine the mechanisms for osteoconductivity the reaction of bone-derived cells (MG63, U2OS, SaoS2, primary human osteoblasts (OB)) to magnesium (Mg) was analyzed. $MgCl_2$ as an *in vitro* model system revealed very heterogenic results. Thus, Mg-based extracts were then applied to converge more realistic Mg degradation environment conditions. Moreover, the influence of direct contact with degrading Mg metal on various parameters was investigated.

METHODS: To determine the influence on proliferation and differentiation the relation between cell count, viability, cell size and the presence of Mg extracts was investigated. For the analysis of direct contact to the material the cells were seeded directly on top of the samples which were pre-incubated for 24 hours in the corresponding cell culture medium.

In addition, expression of genes involved in bone metabolism was determined semi-quantitatively by qPCR.

RESULTS: The cell count of proliferating U2OS and Saos2 is decreased due to extract addition whereas it is increased for MG63 and primary human OB. It is decreased in all cases for differentiating cells. Except for SaoS2 the viability is not influenced. When extract was added primary human OB increased their size. Extract addition led to a bone inducing pattern in primary human OB as well as the direct contact with degrading Mg metal has a strong bone inducing effect.

DISCUSSION & CONCLUSIONS: The results of the cell lines are very heterogenic and declare no specific stimulus of Mg extracts. Anyhow, for primary human OB it is remarkably different. Therefore, it gives the impression that not Mg itself is inducing enhanced bone formation *in vivo* but the combination with other degradation factors.

Once again, it could be proved that as an *in vitro* model for Mg degradation primary cells of the investigated tissue should be used.

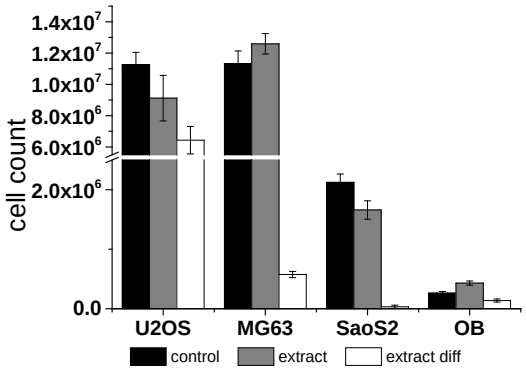

Fig. 1. Cell Proliferation. Cell count of trypsinized cells of the osteosarcoma derived cell lines U2OS, MG63 and SaoS2 and osteoblasts (OB) after incubation under cell culture conditions (control), with extracts or extracts supplemented with differentiation chemicals.

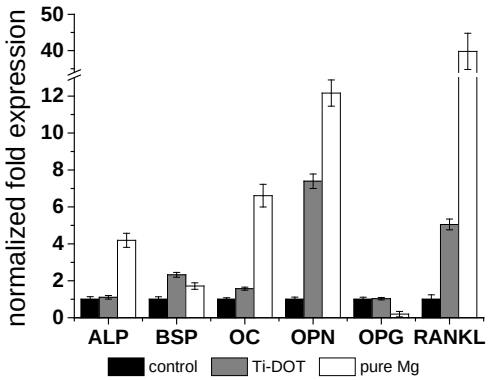

Fig. 2. Semi-quantitative gene expression. Gene expression of genes involved in bone metabolism from osteoblasts after incubation under cell culture condition (control) or on metal samples. Gene expression was normalized to the expression of GAPDH and ß-Actin. GAPDH: glyceraldehyde 3-phosphate dehydrogenase; ALP: alkaline phosphatase; BSP: bone sialoprotein; OC: osteocalcin; OPN: osteopontin; OPG: osteoprotegerin; RANKL: RANK ligand.

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How does blood contact change Magnesium degradation?

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INTRODUCTION: Before any other tissue the first contact of possible implant materials will always be with blood. This will have significant influence on the formation of a first corrosion layer and might determine the fate of the implant in total. In this study we elucidated which are the differences between short-term exposure to whole blood and long-term interaction with plasma on the composition of the corrosion layer. In addition the hemocompatibility of several alloys was studied.

METHODS: Pure Mg, Mg4Y0.5Gd2Nd0.5Dy (abbreviated WE43) and Mg10Gd1Nd (E11) were prepared by permanent mould casting. Cylindrical samples (10 mm x 1.5mm) were cut by electrical discharge machining, cleaned in ethanol and sterilized by gamma-irradiation.

Blood was drawn from 10 healthy volunteers (>20 and <40 years) and anticoagulated using heparin. Samples were exposed to full blood (90 min. and 6 h), as well as blood plasma (72 h) in an *in vitro* "closed loop" chandler-loop system. In hemocompatibility studies coagulation (Thrombin-Antithrombin-III-complex, TAT), number of platelets, white and red blood cells, beta Thromboglobulin, hemolysis, PMN-Elastase, complement system (SC5b-9) and Mg and Ca concentrations in the blood / plasma were determined.

The resulting corrosion layer was analysed by chemical element mapping via electron-induced X-ray emission spectroscopy and the composition by FTIR reflection microspectroscopy.

RESULTS: The amount of Mg in solution showed no differences between the alloys after 90 minutes of blood contact (7-8 mM). After 6 hours treatment this value increased to 20 mM (WE43 and E11) and 26 mM (pure Mg). The immersion in plasma for 72 hours led to lower values (pure Mg: 20 mM, WE43: 15 mM, E11: 10 mM). Calcium-content in solution was generally about 20% higher in whole blood than in plasma. The exposition of the samples to blood had no significant effects on TAT, number of platelets and white and red blood cells, beta Thromboglobulin and hemolysis (except for pure Mg after 6 hours) compared to the control tube without sample. However, the values for PMN-elastase and SC5b-9 were significantly elevated after 90 minutes and increased further after 6 hours.

Chemical element mapping and FTIR revealed distinct differences concerning the corrosion layer in the different fluids independent of the used alloy. During blood contact the main corrosion product was determined to be magnesite ($MgCO_3$), associated with the formation of calcium phosphates, whereas in plasma contact mainly brucite ($Mg(OH)_2$) was the predominant corrosion product.

DISCUSSION & CONCLUSIONS: The clinical parameters indicate that all alloys are influencing the leucocytes towards an inflammatory reaction (increase of PMN-elastase), which could be due to the formation of calcium phosphates [1]. Even more interesting is the activation of SC5b-9, which has intriguing effects on osteoclast recruitment by the expression of osteoprotegerin by reducing their activity [2]. The differences in the corrosion layer composition indicate additionally the major influence of the environmental conditions.

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Preparation and antibacterial properties of Ag-loaded porous coatings on the Mg-3Nd-4Y-0.5Zr alloy by two-step electrochemical method

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INTRODUCTION: The research and development of biomedical Mg alloys as degradable orthopaedic implants has caused serious concern of researchers are mainly inhibited due to their high degradation rates in physiological environment and consequent loss in the mechanical integrity [1]. However, these implant devices are prone to infection, forcing patients back to surgery for repair or replacement [2]. Implant infections in orthopaedic surgery are becoming a serious clinical problem leading to an increase in complicated and costly revision surgery with a high mortality [2]. To date, the most common way to prevent orthopaedic infections is using antibiotics. Unfortunately, drug-resistance turns out to be a serious problem and the micro-organisms that produce orthopaedic infections have become more difficult to kill. Moreover it requires a long and sometimes intensive treatment. Silver has been employed to fight infections and control spoilage. In minute concentrations, silver is highly toxic to germs while relatively non-toxic to human cells [3-4].

METHODS: The aim of this paper is to investigate the element characteristics and antibacterial properties of Ag-loaded porous coatings on the Mg-3Nd-4Y-0.5Zr alloy that prepared by two-step electrochemical method. The pre-coatings were prepared on the Mg-3Nd-4Y-0.5Zr alloy by anodic oxidation, and then the coated samples were immersed in the $AgNO_3$ solution followed by UV irradiation treatment, the Ag-loaded porous coatings were prepared after micro-arc oxidation treatment in the Ag-containing electrolyte finally. The surface topography of the Ag-loaded porous coatings and the amount and chemical characteristics of Ag in the coatings were characterized by using SEM, EDS and XPS. And the Staphylococcus aureus was used to evaluate the antibacterial activity of the coatings.

RESULTS: The results showed that the coatings prepared by two-step electrochemical method contained a high amount of silver, and most of the Ag was present as molten particles with the size less than 100nm. In vitro antibacterial activity tests indicated the composite coating incorporated with silver had efficient antibiotic ability. 98.78% of the Saphylococcus aureus were killed in first day and the antibacterial ratio was as high as 83.91% after 7 days' incubation.

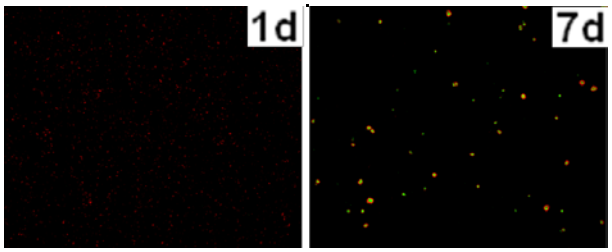

Fig. 1: Images showing viability of the bacteria on samples after 1, 7 days of incubation displayed by fluorescence staining (40X).The live bacteria appear green while dead ones are orange.

DISCUSSION & CONCLUSIONS: The anodic oxidation precoatings have higher ability of incorporating with silver for its higher surface energy. And the amount of Ag introduced to the coatings can make adjustable in certain range by changing the concentration of nitrate solution and soaking time. The Ag-load coatings have standing antibiotic ability mainly due to a lot of Ag nanoparticles was not only on the surface of the coatings, but also embedded in the coatings or fused into the pore walls.

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Excellent mechanical properties and cytocompatibility of non-rare earth Mg-Zn-based alloys

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INTRODUCTION: Mg-based alloys possess remarkable advantages serving as biodegradable implants due to the essential nutritional importance of magnesium for human body, closer elastic modulus to natural bone, and inherent biodegradation capability. Recently, extensive studies focused on the development of Mg-rare earth (RE)-based alloys because this group normally exhibited the highest strength and elongation, and the best corrosion performance [1]. However, some rare earth elements added in Mg-based alloys such as Gd, Y, Pr, Ce, La etc., have been already confirmed to be toxic or have allergic concern [1]. Therefore, it is of great interest to develop non-REs Mg-based alloys with excellent mechanical properties and cytocompatibility.

METHODS: Mg-Zn-X based alloys (X is a confidential non-rare earth element) were prepared and casted under the protection of an argon gas. Solution treatment (T4) was performed at 500°C for 12 hours. Samples after T4 treatment were extruded at 250°C with an extrusion ratio of 10. In-direct MTT test was used to assess the cytocompatibility. Mg alloy extracts (pure Mg and WE43 are used as references) were prepared according to ISO 10993-12. MC3T3-E1 cells were seeded in 96-well plates at 6,000 cells/well. Fresh media was replaced with 100%, 50%, 25% and 10% extract dilutions. After incubating 1 and 3 days, MTT (Invitrogen, US) test was performed. The absorbance was measured at 570 nm using a microplate reader.

RESULTS & DISCUSSION: Fig.1 shows that the Mg-Zn-X alloy exhibits better mechanical properties than pure magnesium and typical Mg-RE-based alloys [2]. Table 1 summarizes the strength and ductility of the alloys from Fig.1. It is clear that Mg-Zn-X alloy exhibits a very good combination of strength and ductility after extrusion. In-direct MTT show that there is decreased cell viability tendency in all groups with the increase of incubation days. In general, Mg-Zn-X shows better or similar viability than the references of pure Mg and WE43.

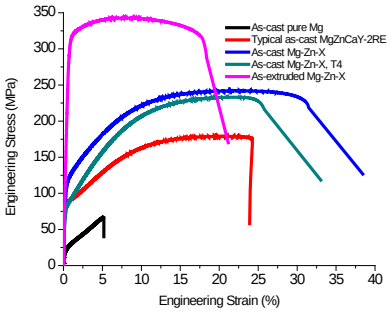

Fig.1: Tensile test of developed Mg-Zn-X alloy in comparison with references.

Table 1. Mechanical properties in Fig.1

Alloys	YS (MPa)	UTS (MPa)	Elonagtion (%)
Pure Mg	28.5	74.3	3.9
MgZnCaY-2RE	81.4	174.6	22.2
Mg-Zn-X	115.4	240.7	26.6
Mg-Zn-X, T4	81.6	233.8	23.2
Mg-Zn-X, extruded	309.6	338.9	14.8

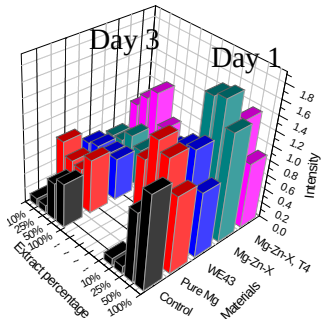

Fig.2: MTT results of Mg-Zn-X alloy and references.

CONCLUSIONS: The newly developed Mg-Zn based alloy exhibits excellent combination of strength, ductility and cytocompatibility compared with pure Mg and commonly investigated Mg alloys, indicating a good candidate for biodegradable implants.

REFERENCES: [1]YJ Chen, ZG Xu, C Smith, et al (under review) *Acta Biomater*. [2] N Zhao, N Watson, Z Xu, et al (accepted) *Plos One*.

Characterization and wear behaviour of MAO on high pressure die cast AM50 magnesium alloy

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INTRODUCTION: Magnesium alloys are promising materials for biodegradable implant applications in orthopedics. However, the usage of magnesium alloys is not yet common due to their poor corrosion and wear resistance [1,2]. Micro-arc oxidation (MAO) has been one among the most preferred treatments to improve the wear resistance of magnesium alloys [1]. In the present study, a silicate-based MAO coating was applied on an AM50 magnesium alloy to enhance its wear resistance. The possible coating formation and wear mechanisms were suggested. The results showed that MAO coating was increasing wear resistance of AM50 magnesium alloy significantly. In conclusion, the MAO coating formed in silicate-based electrolyte represents attractive solution for the biodegradable magnesium alloys.

METHODS: AM50 ingots were molten in an electrical resistance furnace under the protective gas and cast into the step-shape preheated mould. AM50 magnesium alloy discs (Ø16x4 mm) were used as the substrate for MAO process. Samples were oxidized in an aqueous electrolyte containing 15 g/L of Na_2SiO_3 and 2 g/L of KOH with applied voltage 420 of V in the positive and 84 V in the negative half cycle under 20 ± 2 °C. Pulse frequency and duty cycle were 500 Hz and 20%, respectively. Each sample was oxidized for 10 min and then cleaned ultrasonically in acetone and distilled water. Surface morphology and coating thickness of the MAO coated samples was examined by SEM and the phase composition of the samples was identified by XRD. Mean surface roughness (Ra) of the samples was examined by surface profilometry. Hardness of the coatings was measured from their cross-sections by a depth sensing micro-indentation tester using a Vickers indenter. Dry sliding wear tests with continuous measurements were carried out using a ball-on-disk tribometer having alumina ball with a diameter of 6 mm, sliding speed of 10 mm/s and 3 N normal force, respectively.

RESULTS: Fig. 1 shows the surface and cross-sectional morphology of oxidized sample. It is clearly observed that the surface is flat and consists of a large amount of small pores (< 3 μm) with uniform distribution. Some cracks and volcano-shaped structures were detected on the surface. The coating consisted of MgO and $MgSiO_4$ phases and exhibited a thickness of nearly 20 μm.

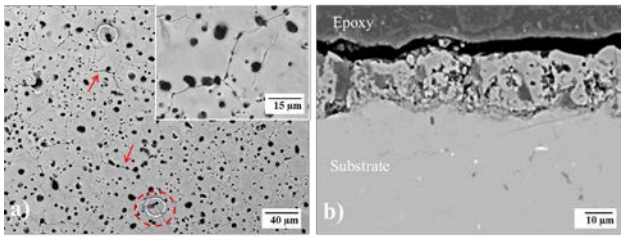

Fig.1: a) Surface and b) cross-sectional images.

Fig. 2 demonstrates the evolution of the friction coefficients of the samples. The wear rate of the MAO sample was significantly higher than that of the bare sample. While the MAO sample exhibited smoother friction coefficient, the bare sample showed unstable friction curve due to severe oxidative wear.

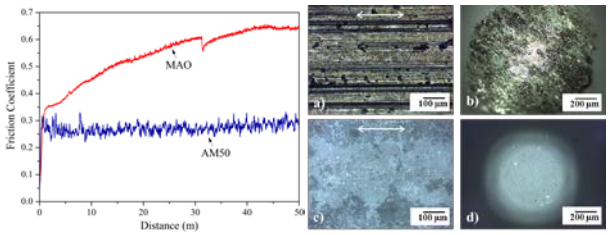

Fig. 2: Friction coefficient of samples and wear tracks and scars on ball.

DISCUSSION & CONCLUSIONS: The MAO coating obtained in this study was found to enhance the wear resistance of AM50 magnesium alloy.

REFERENCES: [1]Y. Zheng, et al (2014) *Mat. Sci.Eng. R* **77**:1-34. [2]F. Witte, et al (2005) *Biomaterials* **26**:3557-3563.

ACKNOWLEDGEMENTS: The authors acknowledge the kind technical collaboration of C. Blawert in HZG.

Development of long-fine WE43 wire for biodegradable medical applications

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INTRODUCTION: Mg alloy-based wire has prospective biodegradable applications such as ligature, stent, sheet mesh, coil, and etc., and the Mg alloy wire is required to be long-fine and to have appropriate mechanical properties for such applications [1-3]. The purpose of this study is to develop a long-fine WE43 alloy-based wire for biodegradable applications, and to investigate the microstructure, the mechanical properties, and corrosion properties.

METHODS: Long-fine WE43 wires with three different diameter sizes (ϕ196 μm, ϕ 98 μm, ϕ 54 μm) were fabricated by conventional drawing process, as shown in Figure 1. A WE43 billet of commercial purity was extruded into wire rod shape (ϕ1.9 mm) at 723 K with an extrusion ratio of 28:1 and was annealed. Then the wire rod was repeatedly cold-drawn and annealed until the wire diameter reduced to the requested size. All the annealing was carried out at 673 K for 30 minutes.

The dimension measurement, microstructural observation, and mechanical testing were made for the as-drawn fine wires to examine the dimension error and microstructures and mechanical properties. The as-drawn fine wires were immersed in a bovine serum at 310 K for 24 hours to examine the corrosion properties.

RESULTS: The fabricated fine wires have a high-accuracy circular shape in cross section and smooth surface (Figure 2), and the diameter errors of all wires are less than 0.1%. Table 1 shows the mechanical properties and corrosion rates of the wires. The ultimate tensile strength of the wire indicates 324 MPa at 196 μm in diameter and decreases with reducing the diameter size, and the elongation of the wire indicates very low values in all diameter sizes. The corrosion rates of the wires with 98 μm and 54 μm in diameters are respectively 0.1 mm/y and 0.3 mm/y, which are lower than that of the wire with 196 μm in diameter, 1.3 mm/y. SEM observation of the immersed samples shows that the wires with 98 μm and 54 μm in diameters are uniformly corroded, unlike local corrosion in the wire with 196 μm in diameter.

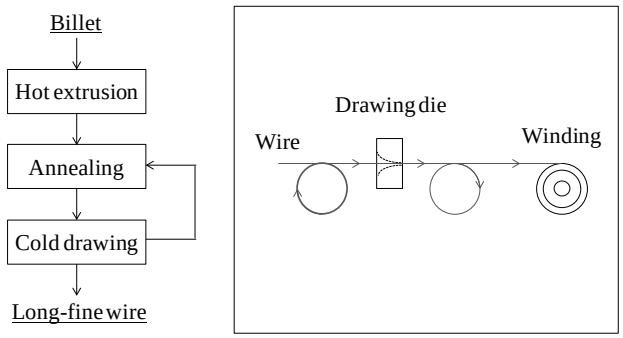

Fig. 1: Schematic diagram of long-fine wire drawing and fabrication process flow.

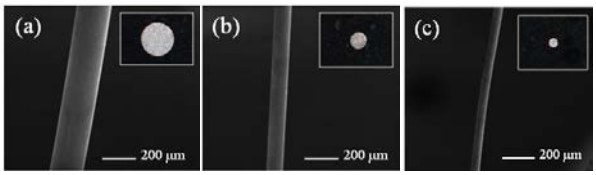

Fig. 2: Appearances of as-drawn WE43 alloy wires with (a) 196 μm and (b) 98 μm and (c) 54 μm in diameters.

Table 1. Mechanical properties and corrosion rates of fine WE43 wires

Wire dia. /μm	σ_{UTS} /MPa	ε /%	Corrosion rate /mm·y^{-1}
196	324	1.4	1.3
98	301	2.4	0.1
54	270	2.5	0.3

DISCUSSION & CONCLUSIONS: The long-fine WE43 wires with different diameters are fabricated by conventional drawing process, and they have high dimension accuracy and very low surface roughness. Reducing the diameter size, the corrosion properties of the wire are improved, although the tensile strength decreases.

REFERENCES: [1] S. Nazari (2012) *Front Lines of Thoracic Surgery* (eds S. Nazari) InTech, pp 263-292. [2] F. Witte (2010) *Acta Biomater* **6**: 1680-1692. [3] Q. Pen, H. Fu, J. Pang, J. Zhang, W. Xiao (2014) *J Mech Behav Biomed Mater* **29**: 375-384.

A comparative study of the degradation of pure Fe and Fe-20Mn-1.2C alloy in modified Hanks' solution for biodegradable cardiovascular devices

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INTRODUCTION

Biodegradable metals for cardiovascular applications are expected to degrade completely upon fulfilling the mission to support tissue during healing without generating toxic effects [1]. Fe-based alloys show promising potential for cardiovascular devices, such as stents [2]. High-Mn austenitic steels (Fe-20Mn-1.2C) show higher yield and tensile strength and plastic deformation compared to pure Fe [3]. This work focuses on the degradation behaviour of pure Fe and Fe-20Mn-1.2C alloy in modified Hanks' solution. *In vitro* degradation behavior of the materials is studied and a corresponding mechanism of degradation associated to the chemical composition of the solution is discussed.

MATERIALS AND METHODS

As-received commercial iron alloys were considered in this study. Modified Hanks' solution [2] was used for static immersion tests (ASTM G31-72). Samples were immersed for 2 weeks in a controlled simulated human body environment. Ion release from the samples was assessed by atomic absorption spectroscopy (AAS). Optical and scanning electron microscopy (SEM), X-ray dispersive spectrometry (EDS) and X-ray diffraction (XRD) were used as characterization techniques. Corrosion rates were calculated with weight loss method.

RESULTS AND DISCUSSION

The degradation layer on the pure Fe was found to be composed mainly of iron and oxygen, with traces of phosphorus, chlorine and calcium. The degradation of Fe-20Mn-1.2C leads to the formation of a thicker and homogeneous $MnCO_3$ deposit on the surface (Figure 1). An adhesive degradation layer is formed on the substrate surface, so that it prevents the contact of the material with the electrolyte. The release of Fe and Mn ions into the solution was limited due to the barrier effect of the insoluble degradation layer compare to iron.

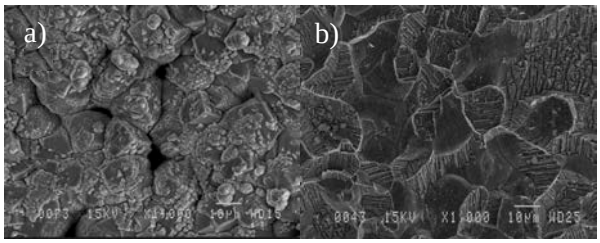

Figure 1: Surface morphology after in-vitro test (a) Fe-20Mn-1.2C and (b) pure iron.

CONCLUSIONS

The results of the present *in vitro* study indicate that the pure Fe and Fe-20Mn-1.2C alloy are suitable biomaterials for degradable implants. Fe-20Mn-1.2C showed a lower corrosion rate compared to that one of pure iron. The presence of a CO_2-rich atmosphere, HCO_3^-, CO_3^{2-}, Cl^-, Ca^{2+} and phosphate ions in the solution play an important role on the degradation/passivation process.

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[1]Y. Zheng et al. (2014) Materials Science and Engineering R 77 1-34, [2]M. Moravej et al. (2011) Materials Science and Engineering: B, 20:1812–1822, [3]O. Bouaziz et al. (2000) Curr Opin Solid State Mater Sci. 15:141-168.

ACKNOWLEDGEMENTS

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Thermodynamic calculation and experimental studies of phase relations in the Mg-Si-Ca-Sr system for degradable biomaterials.

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INTRODUCTION: As a biodegradable material, Mg provides biocompatibility for bone regeneration applications, which makes it a preferred material for new temporary implants. Alloying can improve the properties of Mg. Therefore, the intended aim of this research is to correlate the mechanical behaviour with the microstructure through characterization of several Mg-based alloys.

Ca, Sr and Si were selected to develop magnesium alloys for biomedical application due to their good biocompatibility [1]. Ternary Mg-Si alloys with Ca or Sr and quaternary Mg-Si-Ca-Sr alloy were produced and characterized to investigate their mechanical properties and to relate them to their microstructure.

METHODS: The alloys were prepared using permanent mould gravity casting by melting 99.98% pure magnesium ingots, pure strontium and calcium chips (98.9%, 97.3%), and a Mg10Ca, master alloy. The raw materials were molten in an electrical resistance furnace under the protective atmosphere of argon and SF_6 gas. The produced specimens were cut into disks that were ground and polished with water free alumina solution.

The surface morphology was determined by scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS), Image-Pro was used to measure the grain size. Compression and HV 0.25 Vicker's hardness tests were used to compare the mechanical properties depending on the concentration of alloying elements. The presence of secondary phases and their fractions (vs T) were calculated with the software ThermoCalc® and its thermodynamic magnesium database TCMG2; by X-ray diffraction (XRD), refinemet Rietveld method, EBSD and EPMA the predictions have been compared with the experimental data for each sample.

RESULTS: The presence of the predicted phases at room temperature (Fig.1) was confirmed for Mg-Ca-Si ternary system. For the Mg-Sr-Si system the phase content could be explained by the hypothesis that Ca and Sr form intermetallic phases with the same structure [2-3]. The same assumption has also been applied to interpret the results for the quaternary Mg-Si-Ca-Sr system.

Vicker's hardness tests (Table 1) reveal higher values for the alloys in the Mg_2Ca+CaMgSi+Mg region than for the alloys in the Mg_2Si+CaMgSi+Mg region of the Mg-Ca-Si ternary phase diagram.

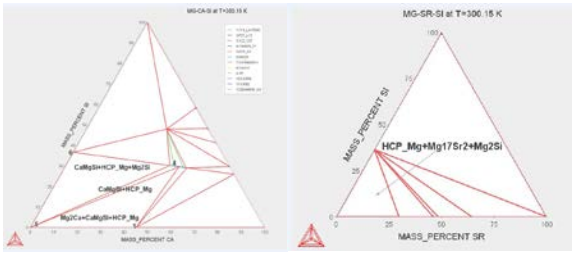

Fig. 1: Isothermal sections at room temperature for the systems Mg-Ca-Si and Mg-Sr-Si calculated with TCMG2 database in ThermoCalc.

DISCUSSION & CONCLUSIONS: The hardness of as-cast Mg-Ca-Si was associated to the fraction of secondary phases, a higher presence of Mg_2Ca. The absence of Mg_2Si appears to correlate with a higher hardness of the alloys.

Table 1. Mass percentages of the intermetallics present in the alloys related to the hadness .

Alloy	% Mg_2Si	% Mg_2Ca	%MgCaSi	HV0.25
Mg2,07Ca0,4Si		4.3	0.5	55.0±4.1
Mg1,9Ca0,76Si		2.9	0.8	52.7±1.2
Mg1,77Ca0,97Si		0.3	6	46.8±3.8
Mg0,88Ca1,54Si	0.6		2	44.1±4.1
Mg0,5Ca1,7Si	1.6		1.1	41.8±9.2
Pure Mg				37.0±2.2

REFERENCES: [1]H.R.Bakhsheshi-Rad et al. (2014) *Materials and Design* **53**:283-292. [2]Y.F.Zheng et al (2014) *Mat. Sci. Eng. R* **77**:1-34. [3]J.W.Bennet et al (2013) *Phys. Rev. Lett.* **110(1):**017603.

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Influence of corrosion on mechanical properties of Mg5Gd wire

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INTRODUCTION: Addition of Gd improves the mechanical properties and corrosion rate of Mg [1]. The Mg5Gd wire studied here could find its application as Kirschner wires for temporary fixation of bone fractures. Due to recrystallization during their complex forming processes, metal wires show fine grained microstructure resulting in high strength and ductility. Since these wires are exposed to bending, before & during implantation the strength influenced by corrosion is of interest.

MATERIAL & METHODS: Wires of a final diameter of 1.6mm were produced from cylindrical gravity castings via extrusion followed by solution heat treatment at 500°C for 6h and wire drawing at 350°C using up to 9 drawing steps with an average deformation degree of 0.3 finalised by an annealing heat treatment. Mechanical testing was conducted on a TIRA28100 universal testing machine. Samples (up to 5) for tensile tests had a length of 50mm, for bending 30mm (span: 25mm, deformation speed: 1mm/min). Compression samples had a ratio of height to diameter of 2:1. Ringer-Acetate solution was used in the corrosion tests due to its similarity to human blood. Three wires were corroded in 500ml electrolyte at 37°C.

RESULTS: The tensile yield strength of Mg5Gd wire is with 290MPa rather high. Due to the notch effect in the clamp area, the maximum tensile strength and elongation cannot be measured. From additional compression tests a tensile-compressive yield asymmetry (Δ100MPa) can be seen (Fig. 1a).

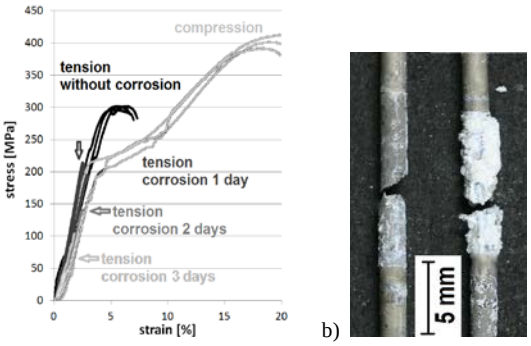

Fig. 1: a) Tensile and compressive stress-strain curves revealing tensile - compressive yield asymmetry and influence of corrosion on tensile strength, b) tensile samples after corrosion of 1 day (left) and 3 days (right)

The bending stress-strain curves in Fig. 2a reveal a significant reduction in bending yield and bending strength as well as ductility after corrosion, even though the bending strength itself is very high for Mg alloys. Once local corrosion takes place (see Fig. 2b and 1b) it causes a notch effect and the material fails due to stress peaks; the distribution of strength values is found to be very high and will depend on the size and shape of the corrosion pits.

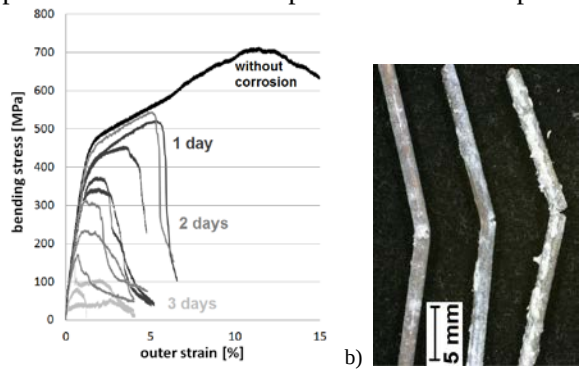

Fig. 2: a) Bending stress - outer strain influenced by corrosion time, b) samples after bending: 1-3d

DISCUSSION & CONCLUSIONS: The high ductility of the wire can only be determined in compression and bending tests and is a result of the fined grain size (~30µm). Corrosion in Ringer solution strongly reduces tensile and bending yield strength due to pitting corrosion. Microstructural investigations reveal twins and Mg-Gd phases, which mostly form in the extrusion and drawing direction. These intermetallics are more novel than the Mg-matrix and cause microgalvanic corrosion. It applies to find out how a solution heat treatment will reduce second phases without affecting the mechanical properties. Further future work is the influence of pre-bending on the mechanical properties and the corrosion behaviour. Since the mechanical properties itself are very good and the acute toxicity of Gd is only moderate [2], but the degradation is not homogenous, a surface coating might improve long term stability.

REFERENCES: [1]N. Hort et al (2012) *Acta Biomaterialia* **6(5)**:1714-25. [2] F. Feyerabend et al (2010) *Acta Biomaterialia* **6(5)**:1834-42

Cyclic voltammetry for prediction of degradation of resorbable metallic biomaterials: possibilities and limitations

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INTRODUCTION: Corrosion rates of metallic biomaterials can be easily assessed by immersion test. To reduce the time for testing of new developed alloy composition and treatments electrochemical measurements are frequently used. Mostly linear sweep voltammetry measurements are performed with different scan rates, up too 10 mV/s. A discrepancy between corrosion rate data from immersion tests as well as from in-vivo measurements and electrochemical data is discussed [1].

The interpretation of electrochemical data bases on the assumption of a "passive" layer, covering the surface. But observations during polarization of Mg or Mg-alloys show variations in the reproducibility and shapes of I vs. E curves.

As well the "passive" curves as the "switch" curves are not adequate for a reliable assessment of the corrosion rate, based on the simple reaction

$$Mg \rightarrow Mg^{2+} + 2e^{-} \qquad (1)$$

3 different reaction pathways are discussed for the oxidation of Mg in contact with aqueous electrolytes [2]. Besides the NDE (negative difference effect) influences the measurements by hydrogen evolution at anodic polarisation. The hydrogen evolution is initiated by galvanic coupled reactions at the surface where the created hydrogen bubbles block parts of the electrochemical active surface.

The question is how the galvanic coupling can be excluded and the calculation of the corrosion rate values based on current measurement around E_{corr} get reliable data.

METHODS: Pure Mg (99,9+), WE 43(HAP), Zn, Al and Y as alloying elements and Fe would measured in Ringer and cell culture solutions using cyclic voltammetry techniques. Polarisation was performed using MCS connected to ECI 1286 potentiostate [3]. Following parameter would be varied: 1. cathodic threshold potential and 2. scan rate. 5 cycles were performed after 15 min of OCP measurement. As well from the forward as the backward scan $E_{I=0}$, i_0,R_p,b_a, b_c and corrosion rate were estimated using corrware software.

RESULTS: In case of Mg and Mg-alloys it is possible to find scan rates where the cyclic I-E curves are "slightly" reversible and the slope can be used for the calculation of the corrosion rate, Fig.1. The application of MCS set-up permits higher scan rates because of the reduced measurement area. During cyclic voltammetry the investigated surface will cleaned and activated. At higher scan rates the hydrogen evolution doesn't play any role and the current flow through the circuit is directly connected to the o.m. reaction and not influenced by any site reactions.

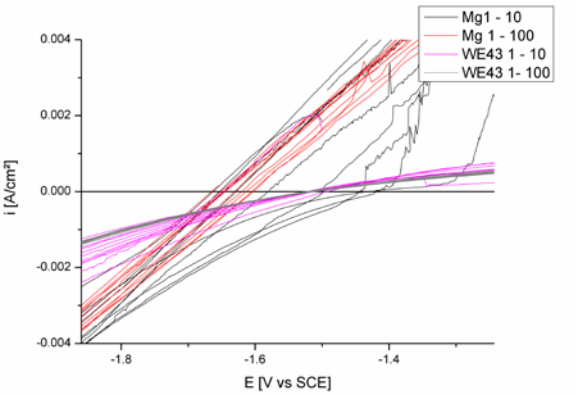

Fig.1 cyclic voltammogram's of pure Mg (black, green) and WE 43 (margenta, blue) in ringer solution performed with 10mV/s and 100mV/s the slope of the curves represent the polarisation resistance used for calculation of corrosion rate.

CONCLUSION: Based on these first preliminary results can be concluded that the cyclic voltammetry is a helpful tool for reliable assessment of corrosion rates of highly active elements, respective alloys based on Mg.

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Electrochemical study of Mg-0.6Ca alloy under a constant strain condition

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INTRODUCTION: Complications rose from the current use of metallic microfixation techniques to address craniomaxillofacial trauma and anomalies in children, i.e. growth restriction and transcranial migration [1], have led to the development of biodegradable plate systems [2]. In this work, biodegradable Mg-Ca alloy is proposed as craniofacial plate material and its degradation under strained condition was studied.

METHODS: Mg-0.6Ca alloy was prepared via casting from Mg ingot and Ca powder (purity 99.99%). The cast samples were cut into flat "dogbone" shape specimens (12 mm gage length, 3 mm thickness, 3 mm width, 6 mm radius). The specimens were ground with SiC paper (1200 grit), and ultrasonically cleaned in ethanol. Potentiodynamic polarization (PDP) and electrochemical impedance spectroscopy (EIS) were carried out in simulated body fluid. Ag/AgCl, graphite, and unstrained/strained specimens were used as the reference, counter and working electrodes. The PDP was done with a scan rate of 0.2 mV/s at ±0.25 V against open circuit potential. EIS was done at frequency range 1 Hz to 10 kHz with perturbation amplitude of 10 mV. Tafel extrapolation and EIS analysis were computed at 30 min, 24 h and 72 h. Strains were induced to the specimens by a 0.5–1 N load range stressing jig throughout the immersion time.

RESULTS: Figure 1 shows Tafel and Nyquist curves obtained for strained specimens after 30 min, 24 h, 48 h, and 72 h immersion. Figure 2 shows the effect of strain on fracture morphology. Figure 3 compares the behaviour of corrosion current and corrosion rate as a function of time between unstrained and strained specimens.

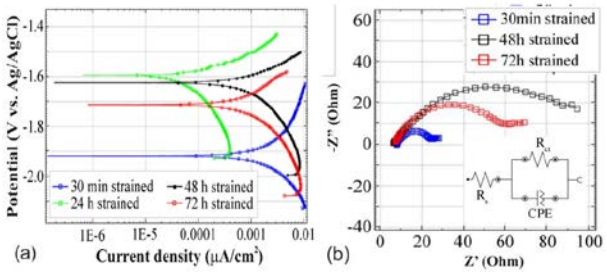

Fig. 1: (a) Tafel and (b) Nyquist curves for strained specimens at different immersion time. Equivalent circuit is shown (inset b).

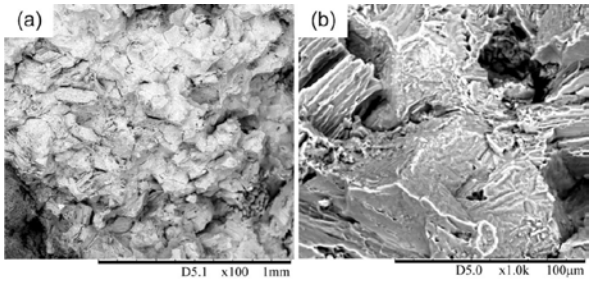

Fig. 2: (a) Fractograph of strained specimen after 72 h immersion, and (b) at higher magnification.

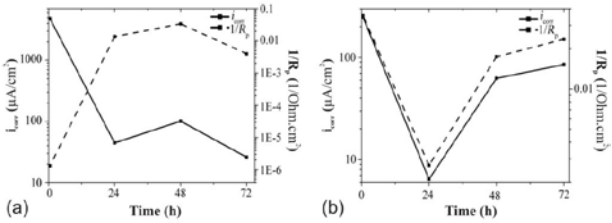

Fig. 3: Corrosion rate (~1/Rp) from EIS and corrosion current from PDP as a function of time for: (a) unstrained, and (b) strained condition.

DISCUSSION & CONCLUSIONS: Degradation behaviour of strained Mg-0.6Ca alloy proposed for craniofacial plate was studied using electrochemical measurements. Potentiodynamic polarization tests measured a fluctuated corrosion current density on unstrained specimens, but a steady increase on the strained ones with prolonged immersion time suggesting the combined effect of mechanical deformation and chemical attack. Evidence of transgranular cracking was observed on fractured specimens. Electrochemical impedance spectroscopy tests measured a decrease of double layer capacitance and polarization resistance on the unstrained specimens, but both were fluctuated for the strained ones with prolonged immersion time indicating a disruption to the stability of oxide layer by the introduced strain. Break down of the oxide layer is mainly attributed to the hydrodynamic attack in unstrained specimens and the effect is raised by the strain.

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ACKNOWLEDGEMENTS: UTM and MOE for FRGS grant #R.J130000.7836.4F123.

Methodical Screening of Corrosion Mechanisms of Iron Alloys for the Manipulation of Degradation Rates

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INTRODUCTION: Biodegradable iron and iron based alloys have been studied and initially were used in cardiovascular stents[1]. A common aspect of literature data on biodegradable iron is a rather low corrosion rate and this is also reported in the first in vivo studies using pure iron. The addition of alloying elements may significantly affect the degradation rates. Hermawan et al. tested various Fe-Mn alloys with 20 – 35 % manganese showing increased corrosion rates compared to pure iron [2]. Further improvements have been achieved by a Fe10Mn1Pd alloy, and Liu et al. recommended Co, W, C and S as alloying elements to increase degradation [3, 4]. Our own results showed that the addition of carbon increases the degradation rate, and small amounts of phosphorus did not have any negative effects neither on the cytotoxicity nor on the corrosion properties [5]. However, all results indicate, that further control of the degradation rate of iron alloys is needed. Thus, in the present work aimed at a systematic utilization of known corrosion mechanisms. Therefore, the material was designed that way that the various corrosion mechanisms could be specifically addressed.

METHODS: Samples with diameter of 10 mm and height of 5 mm were manufactured by mixing carbonyl iron (BASF, mean particle size 4 µm) and the respective ferroalloy powders (Fe_3P, FeSi, FeW, FeN), graphite, or MnS-powders. Furthermore iron alloys with a continuous cathodic phase were synthesized by infiltrating Ag-foams (Alantum Europe, Germany) with carbonyl iron powders. All samples were treated by spark plasma sintering (FCT-HP D 250/1) with varying heating rates and temperatures.The samples were grinded, polished and positioned in an electrochemical measuring cell. As electrolyte a simulated body fluid (after Kokubo [6], 37 °C, pH 7.4) with a TRIS buffer was used. The polarization resistance was detected by using a potentiostat (AMEL) with a measuring rate of 5 mV/s. Finally, the current-density-potential curves were analysed by Stern-Geary method.

RESULTS: The results of the polarization measurements are shown in Fig.1. In comparison to the pure iron reference the lowest polarization resistance levels are detected when silicon in low amounts or tungsten is used as alloying element. Furthermore, rather low polarization resistance is obtained when continuous silver phases are realized. In contradiction, high resistance is measured when silver is used as spot-like precipitation phase only. Only slight effects have been achieved by alloying with carbon or MnS.

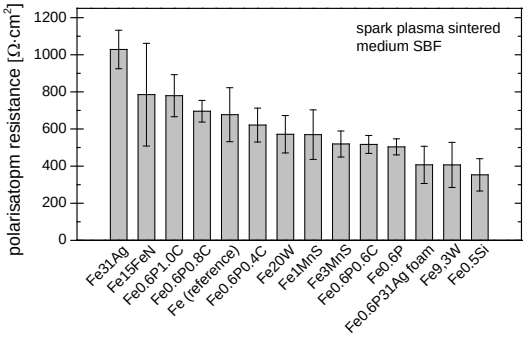

Fig. 1: Polarization resistance of various powder metallurgical iron alloys in simulated body fluid.

DISCUSSION & CONCLUSIONS: General considerations suggest, that a surface corrosion is favourable in order to obtain homogeneous implant degradation. Thus, in particular small amounts of silicon, but also tungsten induce the desired features. Intergranular corrosion also may be addressed by adding carbon or phosphorus. Furthermore, the addition of MnS (which is known to induce pitting corrosion) had no significant effect in the present short-term measurement. Galvanic corrosion only could be realized when continuous cathodic phases have been generated, spot-like precipitations had no significant effect. In conclusion, the results show that the systematic activation of specific corrosion mechanisms may be an appropriate tool in order to taylor the corrosion properties of biodegradable iron implants.

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Bioactive coatings as a key for advanced biomedical applications

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INTRODUCTION: Today there is a demand for short term biodegradable implants. This study will focus on coated and uncoated biodegradable magnesium alloys as well as on the importance of corrosion research and development of in-situ experimental units. Degradable materials such as magnesium and magnesium alloys are of special interest as structural materials, since their high specific strength enables them to be applied as implants. The main problem is that these materials are very reactive and may undergo environmentally induced degradation during service Coatings can address the ability of a controlled degradation biomaterial behavior.

METHODS: Cannulated Interference W4 (96% magnesium, 4% yttrium) screws and their runners which were produced by die casting were investigated under in-vitro (electrochemical and gas formation experiments) and in-vivo (sheep) and (rats) conditions. The coating was obtained by plasma electrolytic oxidation (PEO). The PEO technology is an electrochemical surface conversion treatment .Metal components are exposed to a liquid electrolyte, and an electrical potential is applied to form an oxide-based ceramic. The electrochemical measurements were under 0.9% NaCl solution and corrosion was quantified electrochemically by electrochemical impedance spectroscopy (EIS) .The experimental setup for EIS consisted of a unique bio-reactor system In order to mimic the tissue conditions as closely as possible the solution was adjusted to 36.5–40Cº and pH 7.35–7.45. The flow rate of the solution between the reactor (3000 ml) and the electrochemical cell (500 ml) was chosen to be 100 ml/min.The coating topography was investigated by scanning electron microscopy (SEM) and two in-vivo studies were performed on sheep's femur 6 and 12 weeks and on rat's femur (W4 Pins) 6 and 12 weeks.

RESULTS: The in-vitro measurements showed a significant decrease in corrosion for the W4 PEO coated samples compared to the uncoated samples by up to 90% both in EIS and gas formation. It was also shown that PEO coating of W4 screws resulted in a significant improvement of gas formation in-vivo for the rat's femur after 12 weeks implantation. In order to understand the reason for a bio-active controlled magnesium surface Figures 1 shows a HA coating. The initial degradation represents the occurring bio-degradation of a bio-degradable implant immediately or directly after implantation.

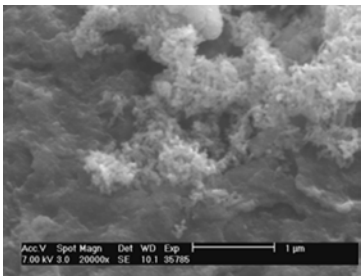

Figure 1: SEM image of the HA coating

DISCUSSION & CONCLUSIONS: 1.Effective tailoring of corrosion by CaP- PEO nano coating for W4 magnesium alloy lead to a reduction of corrosion by up to 90%. 2. The results lead to the conclusion that in order to effectively tailor and control the biodegradation of advanced bio-active magnesium implants, the application of PEO coatings is a very promising method.

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Corrosion protection of biocompatible HA/CaP composite coating on AZ60 alloy

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INTRODUCTION: Magnesium and its alloys have attracted considerable attention as potential alternative to conventional implant materials due to their good mechanical properties, attractive biocompatibility and biodegradation properties. However, magnesium and its alloys do not perform well in practice owing to the low corrosion resistance and involved problems. Calcium phosphate (CaP) biocompatible coatings on the magnesium and its alloys have long been investigated [1-3], but the coating structure is usually porous, which is unfavourable for the corrosion protection. So in this work, sol-gel hydroxyapatite (HA) sealing coating was coated on porous CaP conversion coating to form HA/CaP composite coating on magnesium alloy.

METHODS: AZ60 alloy was used as the substrate material. Porous CaP coating was fabricated by chemical conversion method. Then the CaP coated samples were spin-coated with a dipping gel at 2000 rpm for 30 s and subsequently crystallized at final heat-treatment temperatures of 450°C for 2 h under. In vitro corrosion resistance of CaP coating and HA/CaP composite coating were evaluated by potentiodynamic polarization in simulated body fluid (SBF).

RESULTS: The typical surface morphologies of the CaP coating and HA/CaP composite coating are shown in Fig. 1. It can be observed that the CaP coating has the porous structure with random distributed flakes, while the sol-gel coating could overlay most pores and micro-cracks of the CaP coating. The potentiodynamic polarization curves of the coatings are shown in Fig.2. The composite coating showed better corrosion resistance compared to CaP coating.

DISCUSSION & CONCLUSIONS: Better corrosion resistance of the composite coating could be attributed to the effectiveness preventing corrosion fluid to penetrate through the CaP coating defects to corrode the substrate. In addition, since the HA coating connect with the porous CaP coating by physical interlocking, the porous coating would act as the inner lay to increase the adhesion of the sol-gel coating.

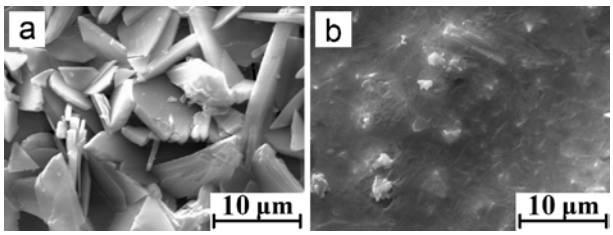

Fig. 1: The typical surface morphologies of the CaP coating (a) and HA/CaP composite coating (b).

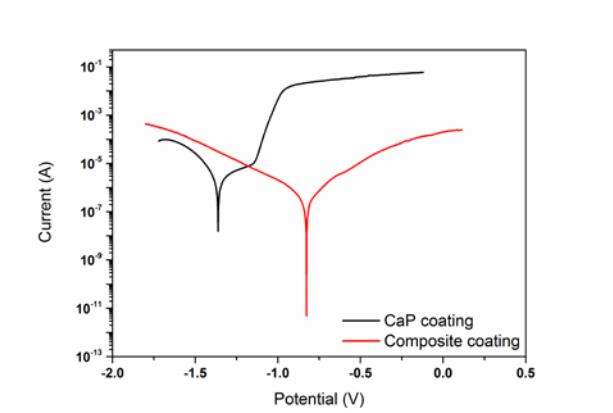

Fig. 2: The potentiodynamic polarization curves of the CaP coating and HA/CaP composite coating in SBF.

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Comparison of the degradation behaviour of binary Mg-Ag and Mg-Gd alloys in PBS and in physiological conditions

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INTRODUCTION: Magnesium and its alloys are widely studied for use as biodegradable temporal implants because of their similar density and mechanical properties compared to bone, as well as their potential non-toxicity, biocompatibility and biodegradability. The required resorption rate depends on the orthopaedic application and therefore different degradation rates are necessary. For this reason, silver (Ag) and gadolinium (Gd) have been selected as alloying elements. Ag accelerates resorption due to the large galvanic potential difference with Mg. Moreover it is used in antibacterial applications [1]. Gd is reported as a slow degrading alloying element in Mg-Gd binary alloys and improves the mechanical properties [2,3]. This work compares behaviour of Mg-Ag (2w%, 4w%, 6w%) and Mg-Gd (5w%, 10w%, 15w%) with known materials such as pure Mg and WE43 in two media.

METHODS: Samples are cast at 680-720 ˚C in neutral atmosphere (Ar + SF_6), extruded at 370-450˚C from 30-110mm to 12mm and machined into discs (10x1.5mm) in order to obtain a fine and homogeneous grain size and microstructure. A comparison of the degradation behaviour *in vitro* is given under different testing conditions: phosphate buffered saline (PBS) in air at 37˚C, and Dulbecco's modified eagle medium (DMEM), in air with 5% CO_2 at 37˚C. Hydrogen generation and mass loss in immersion tests are compared with polarization methods. Mass loss results under cell culture conditions (CCC) (DMEM with 10% fetal bovine serum (FBS), 37°C, 20% O_2, 5% CO_2, 95% relative humidity) are also compared.

RESULTS: Each testing condition leads to different degradation behaviour. *Figure 1* shows the resorption rates determined by mass loss under three different conditions. In DMEM all the alloys reach a steady rate after 1 to 2 days of immersion with a degradation rate between 2.8 to 9.3 mm/year. In PBS, however, pure Mg and Mg-Gd alloys up to a 10w% Gd degrade slowly (0.3-0.6 mm/year), while Mg-Ag alloys degrade fast (12.5-17.9 mm/year). Polarization experiments show higher current densities in DMEM (1.4 x 10^{-4} A/cm^2) than in PBS (2.7 x 10^{-5} A/cm^2) increasing with the presence of HCO_3^- as Gulbrandsen concluded [4]. However, the resorption rates in CCC are slower comparing to DMEM (0.2 – 1.1 mm/year), showing the effect that FBS and the control of O_2 and humidity can have.

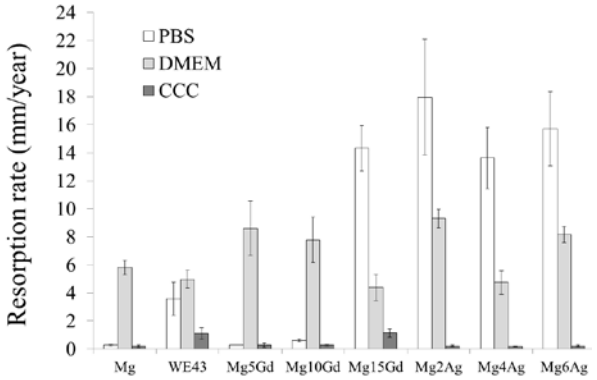

Fig.1 Resorption rates determined by mass loss in PBS, DMEM and CCC.

DISCUSSION & CONCLUSIONS: The results indicate pure Mg, Mg5Gd and Mg10Gd with low amount of impurities and fine microstructure as a slow degrading material and suitable candidates for biodegradable implant. This work shows how much the degradation behaviour is influenced by physiological testing environments which should give a closer comparison with *in vivo* results.

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Study of magnesium fluoride and self-assembled organosilane coatings of AZ31 and MgCa0.8 alloys

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INTRODUCTION: Lately, biodegradable metal such as Magnesium (Mg) and its alloys have been investigated for biomedical applications [1]. Corrosion of Mg and its alloys results in hydroxyl ions, hydrogen gas, dissolved Mg ions and insoluble products such as oxides and hydroxides [2, 3]. *In vivo* rapid corrosion of Mg leads to the formation of gas pockets in the vicinity of the devise [1]. Song et al [4] suggested a rate of 0.01 ml/cm^2/day as an estimate of H_2 evolution tolerance in the human body. The goal of this work was to study the ability of hybrid MgF_2/ organosilane coatings to control the corrosion rate of Mg alloys, fluoride and organosilane coating.

METHODS: Two Mg alloys AZ31 and MgCa0.8 were tested in this study. The alloy samples were 6 mm in diameter and 1 mm thick. The samples for each alloy were divided into four groups: non coated, MgF_2 coated, silane coated and a MgF_2/-silane coated. The MgF_2 coating was conducted as follows. Initially the samples were cleaned with ethanol and boiled in a sodium hydroxide solution (C_{NaOH} = 200 g/l) for 2 h to form $Mg(OH)_2$ layer. The samples were then immersed into a hydrofluoric acid bath (HF 40%) for 96 hrs to convert the $Mg(OH)_2$ layer into a MgF_2 layer.

$$Mg(OH)_2 + 2HF \rightarrow MgF_2 + 2\,H_2O \qquad (1)$$

The silane coating layer was prepared by the sol-gel method as described elsewhere [5-6]. In our study, alkyltriethoxysilanes with alkyl chain lengths n=10 (CnH_{2n+1}Si(OEt)3;CnTES) were used. The precursor solution was prepared as follows: 0.25 ml of 0.01 M HCl was added to a mixture of 2.0 ml ethanol (EtOH), 0.25 ml C10TES, and 0.43 ml Tetramethoxysilane (TMOS). The precursor solution was stirred for 24 h at room temperature to complete the hydrolysis. Mg alloy discs were dip coated in the precursor solution for 1 minute and air dried for 10 minutes at 25 ± 2 °C. A single dipping led to the formation of a uniform 1 micron thick organosilane on the metal surface. The last group was coated with MgF_2 and then followed by the organosaline coating. To assess the rate of corrosion, the H_2 evolution was measured for 120 hours. For this procedure the samples were placed into a beaker with 10 l of 0.9% NaCl. H_2 was collected in a conical flask. Temperature, the H_2 evolution and the pH were measured every 24 h. A constant flow of the fluid was established to prevent changes in the fluid composition during the experiment. The samples were weighted prior and after the H_2 evolution experiments to assess the material loss.

RESULTS: After 120 hrs, the amount of H_2 released by uncoated AZ31 alloys was significantly lower than for the MgCa0.8 alloy. Coating with MgF_2 led to a reduction in hydrogen release. However silane coating led to an increase in the H_2 release although this change was not significant. When coated with both MgF_2 and organosilanes a significant reduction in the H_2 release after 120 hrs was observed for both alloys.

DISCUSSION & CONCLUSIONS: In this *in-vitro* study different coatings were investigated for the AZ31 and MgCa0.8 alloys. The hybrid coating MgF_2/oreganosilane has shown the greatest reduction in H_2 release, indicating that it provides for superior corrosion control properties.

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Influence of different surface modifications of the magnesium alloy WE43 on the electrochemical behaviour

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INTRODUCTION: A drawback of magnesium alloys in biomedical use is their fast degradation. To overcome this problem, coatings can be an appropriate option. Different coatings as well as application methods have been tested in vitro and in vivo with various magnesium alloys (e.g. AZ91, AZ31, Mg-Ca) [1]. In electrochemical corrosion studies most coated magnesium alloys showed an increase in polarisation resistance R_P and lower corrosion current densities i_{CORR} and/or a shift to higher E_{CORR} potentials. A promising coating consists of fluoride treatment in HF acid, however, it is hazardous in use. To avoid HF-coating other fluoride compounds (NaF, KF and NH_4F) can be used. Other strategies to avoid the use of HF are pretreatments, such as cooking in water, NaOH or other acids [2-4]. Compared to other Mg alloys, WE43 demonstrated a good corrosion resistance [5]. The aim of this study was to investigate the influence of different surface pretreatments of WE43 on the electrochemical corrosion behaviour.

METHODS: For this study, each 5 specimens of WE43 (Mg-RE-Zr) were prepared for electrochemical corrosion measurements. The following surface modifications were applied. Group 1: Uncoated samples (grinded with SiC 1200 in ethanol, ultrasonically cleaning in ethanol for 5 min). This type of preparation was the starting point for all modifications. Group 2: Cooking in water at 100 C for 20 min, after drying in air for 20 min immersion in 0.5 M NaOH for 20 min, air drying. Group 3: Cooking in water at 100 C for 20 min, after drying in air for 20 min immersion in 0.02 % oxalic acid for 3 s, cleaning with water, drying by nitrogen gas pressure. Immersion in 0.3 M NaF for 2 h. Group 4: Immersion in 0.3 M NaF for 2 h, drying in air [3,6]. With all surface modifications open-circuit-potential measurements over 1 h followed by R_P measurements (± 20 mV vs E_{CORR}) and cyclic polarisation measurements (CPM; E-Start: -150 mV vs E_{CORR}, E-reverse: -1200 mV vs Ref, E-End: -1700 mV vs Ref, v= 1 mV/sec) were performed in PBS^- (D-PBS, 14190-169, Gibco) heated at 37 °C using a potentiostat (PAR 273, M352/M270, EG&G).

RESULTS: As shown in Fig. 1 all surface modifications reached E_{CORR} values of about -1800 mV after 1 h, in the beginning the E_{CORR} values were different.

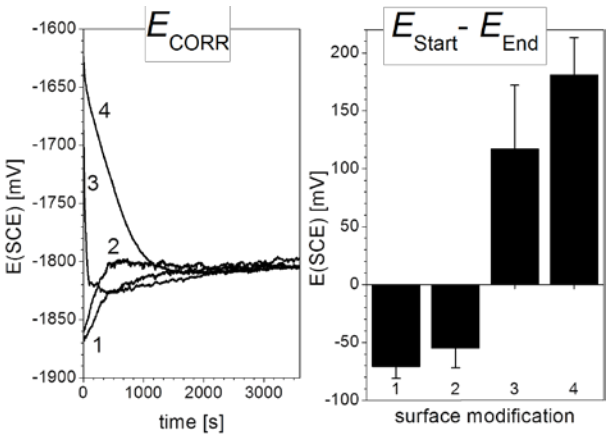

Fig. 1: E_{CORR} mean curves and shifts over 1 h.

The ranking sequence of R_P values of the different surface modifications was: group 2 (3933 ± 17) followed by group 3 (3459 ± 18), group 4 (2616 ± 9) and group 1 (2292 ± 11) Ohm·cm². Compared to the grinded surface the i_{CORR} values from CPM decreased only slightly (group 1: 9 ± 1; group 2: 7 ± 1; group 3: 6 ± 0; group 4: 4 ± 0) µA/cm².

DISCUSSION & CONCLUSIONS: With pretreatment itself (group 2) and, pretreatment plus NaF-coating (group 3) the corrosion resistance of WE43 can be improved. The chosen pretreatment (group 2) can be a good alternative instead of fluoride-coating. In summary, based on these tests, the effect of surface modifications was low compared to the untreated surface.

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Different approaches for *in vivo* testing of absorbable metals in blood vessels

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INTRODUCTION: Before biodegradable metals can be considered as cardiovascular stent material, several implant design and performance criteria need to be investigated. The correlation between *in vitro* and *in vivo* experiments is not predictable [1]. As various alloys, coatings and processing routes can be considered in the construction of an optimal stent, a large number of *in vivo* experiments is necessary to evaluate new materials. Pierson et al [2] proposed a quick and economical method to test new potential stent materials by surgical implantation of wires into the wall and the lumen of blood vessels. In a first trial, we implanted wires using a similar approach. Subsequently we developed a new *in vivo* testing method where the wires of interest were sewn onto an inert polymer carrier stent followed by minimally invasive implantation. This new method was compared to surgically implanted wires.

METHODS: Magnesium alloy rods (Mg10Gd) were hand- or machine-drawn to a diameter of 0.4 mm followed by alkaline cleaning or acidic etching. For surgical implantation, wires were cut to a length of 43 mm and implanted into the left and right femoral arteries of two sheep using sheathed cannulas. For our new carrier stent implantation, wires were cut to a length of 35 mm and sewn on polyamide casted stents (three wires per stent, see Fig. 1). The stents were implanted into the abdominal aorta of two sheep. One animal also received a stent in the carotid artery, which also served as access point for the minimally invasive implantation via Seldinger technique. After explantation, samples were analyzed using MRI and µCT as well as histology.

RESULTS: All implantations were successful. However, several limitations occur when wires are implanted surgically. The number of vessels easily accessible is limited. One animal received two wires per side at different time points. Scar tissue made the re-access of the vessel challenging. With the surgical approach it was not possible to confirm that the wires were in contact with the vessel wall. Ex-vivo imaging showed fragmented wires. In contrast, all implantations of carrier stents were performed without adverse events. The wires were pressed against the vessel wall due to the radial force of the stents.

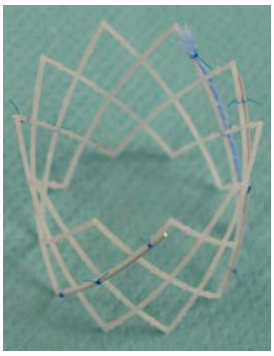

Fig. 1: Carrier stent with three wires sewn on.

DISCUSSION & CONCLUSIONS: Surgical implantation of wires is a valuable but limited method for *in vivo* material testing. It is difficult to consistently position the wire against the vessel wall. As the environment of the wire changes at the vessel entry point, different corrosion rates are obtained along the wire. There are a limited number of vessels easily accessible. Scarring at the surgical site further restricts the applicability.

The carrier stent represents an adaptable and reliable method for *in vivo* material testing. It can be equipped with wires of different materials or coatings. The wires can be placed on the inner or outer surface of the stent, or between the struts. Nearly all vessels can be reached. This allows *in vivo* testing both in arterial or venous blood. As the intervention is minimally invasive, it can be carried out in the same animal several times. This allows implantations in different vessels, at different time points or of different wires in the same vessel, thus reducing the number of animals needed.

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ACKNOWLEDGEMENTS: We thank Dr. Norbert Hort from the Helmholtz Zentrum Geesthacht for supplying the Magnesium alloy. We thank Bernhard Schnackenburg from Philips GmbH Healthcare for selecting and adjusting MRI sequences.

Effects of laser treatment for surface structuring on AZ31 Mg alloy degradation

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INTRODUCTION

Mg-based alloys are highly appealing for degradable biomedical implants and have the potential to substitute some of the current metals employed in orthopaedic and cardiovascular applications [1]. They show a high corrosion rate in physiological fluids [2]: Modulating their degradation pattern and corrosion rate still represents the main challenge to justify their use *in vivo*. A possible way to modulating the corrosion rate is through the modification of the surface morphology and chemistry. This work is aimed at exploring the potential of using laser surface structuring on AZ31 Mg alloy for controlling degradation behaviour. The corrosion rates of different surface structure conditions are assessed.

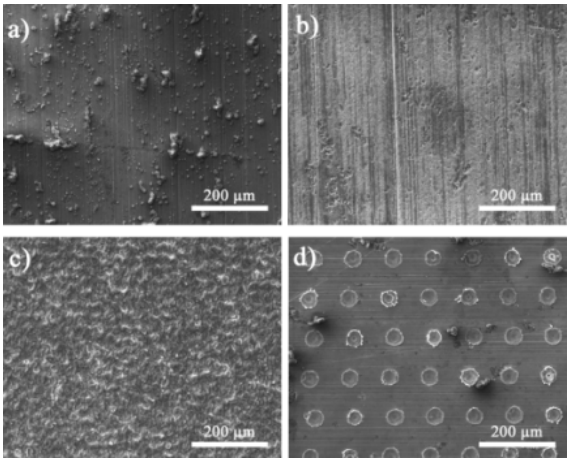

Fig. 1: Tested surface structures a) plain; b) low roughness, c) high roughness, d) dimpled.

METHODS

AZ31 plates (0.4-mm-thick) were cut in 10×20 mm² specimens. Laser surface structuring was performed and 3 structures were patterned: 1- high roughness – HR (R_a= 1.069 µm), 2- low roughness – LR (R_a= 0.218), and 3- dimpled – DP (R_a= 0.862 µm) (Fig. 1). In order to assess the degradation behaviour, static immersion tests was carried out in PBS solution for 14 days. The average corrosion rate (CR_a) was determined based on the weight loss method. Cross sections of the specimens allowed observing the degradation layer. Atomic absorption spectrometry was employed to measure the release of Mg^{2+} ions in the degradation solution. X-ray photoelectron spectroscopy (XPS) analysis was carried out to assess the surface chemistry of all samples before and after the degradation test.

RESULTS

Preliminary results confirmed that surface structure influences the average corrosion rate of the AZ31 Mg alloy (Table 1). The lowest average corrosion rate (1.9 ± 0.03 mm/y) was observed for the DP surface. The thinner degradation layer (40 µm) was observed on the DP samples, whereas for the others specimens it was about 80 µm. Despite these differences, the surfaces showed similar ion release behaviour in the degradation solution. Surface analyses showed that the concentration of carbon atoms on the surface was lower on the surface structured specimens compared to the plain surface before immersion, thus highlighting the cleaning effect of surface treatment. Moreover, LR and HR treatments decreased surface Zn concentration, compared to the amount of the bulk alloy, while DP treatment increased it.

Table 1: Average corrosion rates (CR_a) of the plain and laser structured samples.

Type	Plain	LR	HR	DP
CR_a (mm/y)	2.4±0.03	2.6±0.05	2.3±0.04	1.9±0.03

CONCLUSIONS

The hindered corrosion rate for DP type is suspected to be mainly due to changes in surface chemistry. The formation of degradation layer also followed the dimple geometry, depicting the geometrical influence. The laser treatment can be further optimized for graded corrosion behaviour of Mg-based alloys.

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Combinatorial corrosion studies of Mg-Zn alloy coatings

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INTRODUCTION: Combinatorial technique has drawn attention as a new efficient method for searching and screening new materials [1]. Using combinatorial approach, processing time could be shortened dramatically over the conventional approach. In the present study, the benefit of combinatorial technique in screening the corrosion properties of binary alloy libraries is demonstrated. Mg-Zn binary alloy system is selected as a model for our combinatorial method development. Mg and Zn have significantly different corrosion properties, therefore their alloys can be used for development of coatings with tunable corrosion rates.

METHODS: AJA international 1800F magnetron sputtering system was used for preparation of combinatorial samples. The Mg and Zn were DC sputtered at room temperature in argon atmosphere at a working pressure of 3mTorr. 3 by 1 in glass slides were used as substrates. Energy dispersive X-ray spectroscopy (EDX) was used for the mapping of the chemical composition. The magnesium content varied from 15 at% to 85% across the entire length of the substrate. One of the prepared samples was annealed at 200°C in argon environment to study the effect of heat treatment on the phase structure. X-ray diffraction was used for the identification of different phases in the combinatorial sample. To evaluate the corrosion behavior of the combinatorial samples, a novel optical screening technique was developed.

RESULTS: Fig 1 shows the 2D-XRD map of the as-deposited and annealed samples. XRD patterns of as-deposited sample have only strong Mg (001) and Zn peaks at Mg content of >55 and <33at.% respectively and semi-amorphous and amorphous coating in the rage 42-55at.% of Mg. It is evident that, annealing led to the formation of 8 phases (2 unknown). At low concentration of Mg (i.e. 15-27 at. %), Zn and Mg_2Zn_{11} phases were observed. $MgZn_2$ and Mg_4Zn_7 phases were formed between 27 - 43 at. % of Mg content. At Mg content > 43 at. %, MgZn and Mg phase were observed. By annealing the sample, the zinc rich region became oriented along (001) and magnesium rich regions formed highly textured phases.

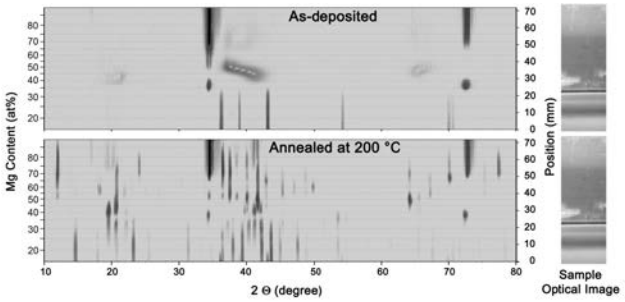

Fig 1. 2D XRD map of combinatorial

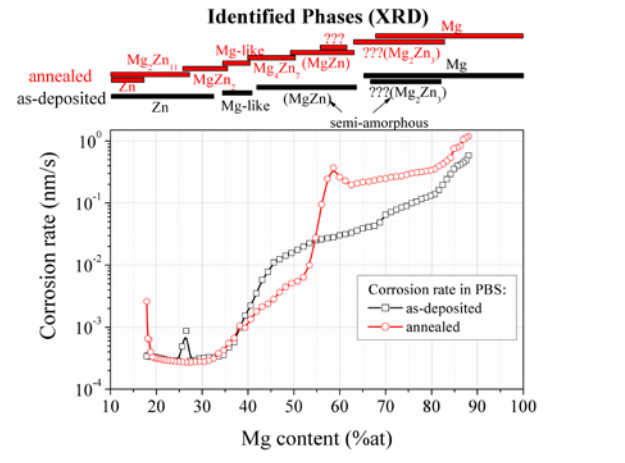

Fig 2. Corrosion rates of combinatorial samples

DISCUSSION & CONCLUSIONS: Several important conclusions can be drawn from the combinatorial corrosion analysis: (i) increase of Zn content in Mg-Zn system in the range 15-85 at% changes apparent corrosion rates by 3 orders; (ii) at Mg content ~60%, annealed (crystallinic) sample corrodes up to 10 times faster than corresponding as-deposited (semi-amorphous) compositions, (iii) at Zn content 40-50% annealed sample corroded slower than as-deposited sample. It is show that crystallites of $MgZn_2$ are very stable.

REFERENCES: [1]McFarland, E.W. and W.H. Weinberg (1999) *Biotechnol* **17(3)**:107-115.

ACKNOWLEDGEMENTS: The authors acknowledge the financial support of the NSF ERC-RMB (NSF ERC 0812348).

In vitro study on the modified ZK60 alloy with the addition of 0.2wt.% Ca

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INTRODUCTION: As very promising biodegradable metals, the main challenges for the wide biomedical application of magnesium and its alloys lies in retarding their high degradation rates in physiological environment and enhancing their biocompatibility. In the present study, industrial ZK60 alloy is tried to be modified with the addition of Ca in order to obtain the combination of good mechanical property, corrosion resistance and biocompatibility.

METHODS: ZK60-0.2Ca alloys were prepared from high-purity Mg (99.95%), Zn (99.9%), Ca (99.9%) and Mg–20Zr (wt%) master alloy. The microstructure and constituent phases were observed by metallurgical microscope and measured by XRD respectively. The mechanical properties were investigated through the tensile test. The corrosion behavior in Hank's solution were characterized by immersion test, and electrochemical measurement. The L-929 cells were used to evaluate the cytotoxicity of the alloy with indirect assay.

RESULTS: The constitutional phase of the ZK60-0.2Ca alloy was α-Mg and Mg_4Zn_7, as shown in *Fig. 1*. The ultimate tensile strength and yield strength of ZK60-0.2Ca were 72.02±14.60 MPa and 117.65±15.62 MPa, respectively, while the elongation was 3.28 ± 0.35%. Corrosion rates calculated from mass loss, ion release and electrochemical measurement were 0.176±0.018 mm/yr, 0.121±0.005 mm/yr and 0.187±0.050 mm/yr, respectively, which were quite close. The cell viability was determined with extracts for 1, 2, 4 days and the results were shown in *Fig. 2*. With the 100% extract in culture medium, the ZK60-0.2Ca showed quite lower cell viability which means that the ions concentration of ZK60-0.2Ca was too high for culturing L-929 cell line. After diluted extract to 10%, the degree of toxicity was almost grade 0 according to ISO 10993-5:2009.

DISCUSSION & CONCLUSIONS: The degradation rate of currently-reported biodegradable magnesium alloys ranged from 0.1 to 2.5 mm/yr [1]. ZK60-0.2Ca is among the group with best corrosion resistance. Moreover, the mechanical properties and corrosion resistance can be further improved through hot working and surface modification. Thus ZK60-0.2Ca can be regarded as a promising biodegradable materials.

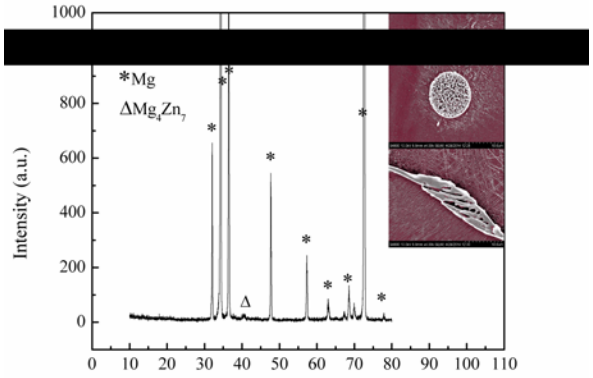

Fig. 1: The XRD patterns of as-cast ZK60-0.2Ca. Inserts are SEM images of the second phase.

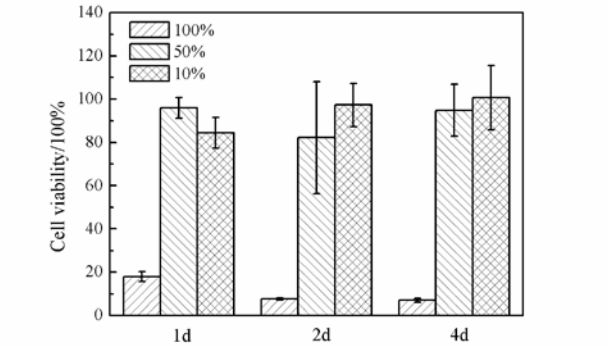

Fig. 2: The cytotoxicity of as-cast ZK60-0.2Ca to L-929 cells with different concentrations of the extracts.

REFERENCES: [1]N. Li and Y.F. Zheng (2013) *J Mater Sci Technol* **29**:489-94.

ACKNOWLEDGEMENTS: This work was supported by the National Basic Research Program of China (973 Program) (Grant No. 2012CB619102 and 2012CB619100) and National Science Fund for Distinguished Young Scholars (Grant No. 51225101).

Influence of binary magnesium alloys corrosion on morphology and adhesion of human undifferentiated cells

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INTRODUCTION: In orthopaedics there is an increasing attention on magnesium as resorbable biocompatible materials for bone regeneration. However, rapidcorrosion rate of magnesium induces the loss of mechanical properties, whichhindersproper bone healing *in vivo* [1]. Several alloy elements, such as silver and some rare earth elements, have been combined with commercially pure magnesium to modulate corrosion properties and mechanical strength in order to temporary support the healing of the injured bone tissue and allow proper cellular growth.However, not much is known about the detailed cellular interactions. Thus, in this study, the corrosion properties of Mg2Ag, Mg10Gd and WE43 alloys were analysed under cell culture conditions. Furthermore, the short-term cell response of human umbilical cord perivascular cells (HUCPVC) was investigated in terms of cell morphology and adhesion.

METHODS: Discs of three different magnesium alloys, Mg2Ag (1.89% Ag, Mg Bal.), Mg10Gd (8.4% Gd, Mg Bal.) and WE43 (3.45%, Y, 2.03% Nd, 0.84% Ce, Mg Bal.) were used in this study. Commercially pure magnesium (99.97% Mg) was used as control. Mg alloy discs (1cm diameter, 0.2g average weight) were produced by gravity casting followed by a T4 heat treatment. Afterwards, discs were extruded at 370°C using speeds between 3 and 4.5 mm/sec. Commercially pure Mg (cp Mg) discs with the same dimensions were cast by direct chill and extruded at 300°C and 0.7 mm/sec. Corrosion parameters were analysed after 24h, 48h and 72h of incubation in culture medium by means of mass loss method, cryoscopicosmometer, pH-meterand atomic force microscopy (AFM). Changes in cell morphology and adhesion dynamics were evaluated culturing human umbilical cord perivascular cells (HUCPVC) on corroded materials using scanning electron microscopy (SEM) and actin cytoskeleton/focal adhesion staining.

RESULTS: It was revealed that the pH and osmolarity of the medium increased with increasing corrosion rate and thiswas found to be more pronounced for WE43 alloy.

For all the samples, the surface roughness was higher after 3 days of corrosion due to their pitting tendency in culture medium.

The biological investigation showed that HUCPVC exhibited higher density on Mg2Ag and Mg10Gd due to the lower alkalinization and osmolarity of the medium. However, cells grown on WE43 generated more developed and healthy cellular cytoskeleton, which allowed them to better adhere to the surface.

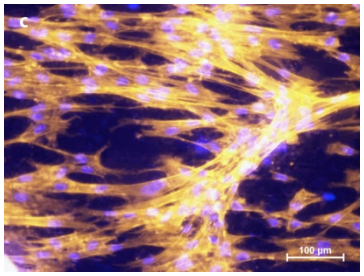

Fig. 1: Confocal fluorescence microscopy of focal adhesion and actin cytoskeleton in HUCPV cells cultured for 24h on 24h pre-incubated WE43 samples. Monochrome images at 20x were overlaid displaying the triple labeling.

DISCUSSION & CONCLUSIONS: HUCPVC adhesion and spreading are likely reduced on WE43 due to the increased pH and osmolarity associated with the higher corrosion rate. However, cells, morphologically, exhibited a more healthy state when grown on WE43, developing more solid cytoskeleton along each direction. This allows the correct cell initial recruitment and adhesion when placed in living tissues.

REFERENCES: [1] Witte F, Kaese V, Haferkamp H, Switzer E, Meyer-Lindenberg A, Wirth CJ, et al (2005) *Biomaterials* **26**:3557-63.

ACKNOWLEDGEMENTS: The research leading to these results has received funding from the People Programme (Marie Curie Actions) of the European Union´s Seventh Framework Programme FP7/2007-2013/ under REA grant agreement n°n289163.

In vitro study on biodegradable Mg-1Ca-2Sr-XZn (X=2, 4, 6) alloys

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INTRODUCTION: The current magnesium and its alloys degrade quickly and their corrosion resistance properties need to be further improved. Among the methods to improve the corrosion resistance of magnesium, alloying is a very effective way. Taking both the biocompatibility and the corrosion resistance property into consideration, alloy element such as Calcium(Ca), Strontium(Sr) and Zinc(Zn) were selected to fabricate Mg-1Ca-2Sr-xZn(x=2,4,6) alloys. In our present work, the microstructure, phase composition, mechanical properties, electrochemical properties in Hank's solution and cytotoxicity in vitro of these three kind of as-extruded alloys were studied.

METHODS: Commercial pure Mg (99.9%) and Ca, Sr, Zn(99.5%) metal ingots were used as raw materials. After solution treated at 340°C for 4h, the as-cast alloys were extruded at the temperature of 325°C at an extrusion rate of 2mm/s. The microstructures of as-extruded alloys were examined by optical microscopy after etched. X-ray diffraction analysis (XRD) was used to examine the phase composition. The Tensile tests were carried out with an Instron-5969 universal testing machine at a constant crosshead speed of 1.00mm/min at room temperature. Electrochemical tests were conducted in Hank's solution at 37°C. MC3T3-E1 cells were adopted to evaluate the cytotoxicity by the indirect assay.

RESULTS: XRD data released that the Mg-1Ca-2Sr-xZn(x=2,4,6) alloys mainly composed of α-Mg, Mg_2Ca and $Ca_2Mg_6Zn_3$ phase. These secondary phases were found to be distributed both inside the grains and along the grain boundaries (Fig.1). Mechanical properties of experimental alloys were depicted in Fig. 2. Both the YS and UTS increased with the increasing Zn contents, while the Mg-1Ca-2Sr-4Zn alloy exhibited the significantly highest elongation rate about 20.91%. Moreover, Mg-1Ca-2Sr-4Zn alloy exhibited the lowest corrosion current density about 1.315±0.079 μA/cm^2. The Mg-1Ca-2Sr-4Zn alloys exhibited Grade I toxicity even after 7 days' incubation by indirect assays. ALP tests showed that Mg-1Ca-2Sr-4Zn alloys exhibited higher ALP activity than other two alloys.

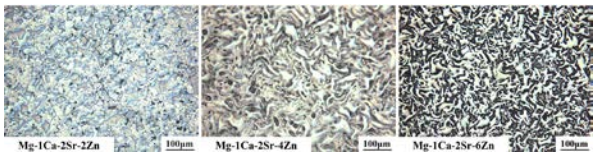

Fig.1 Optical microstructure of experimental alloys

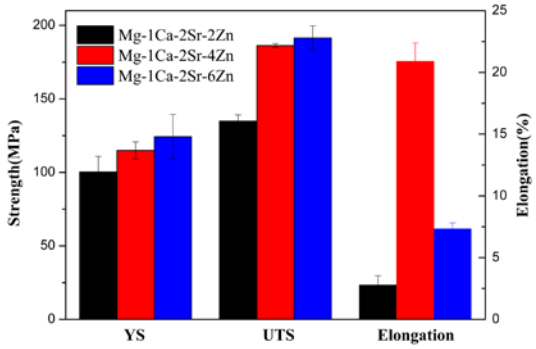

Fig. 2 Mechanical properties of experimental alloys

DISCUSSION & CONCLUSIONS: The increasing Zn contents in the alloys can both refine the grain size and increase the amounts of secondary $Ca_2Mg_6Zn_3$ phase. Among the three alloys, Mg-1Ca-2Sr-4Zn alloys exhibited the best mechanical property, corrosion resistance property and in vitro biocompatibility, and would be a potential biodegradable metal for orthopaedic applications.

ACKNOWLEDGEMENTS: This work was supported by the National Basic Research Program of China (973 Program) (Grant No. 2012CB619102 and 2012CB619100) and National Science Fund for Distinguished Young Scholars (Grant No. 51225101).

Application of proteomics for investigating the impact of magnesium implants on bone tissue

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INTRODUCTION: Information about changes of the composition of proteomes in relationship to defined perturbations can provide insight into the mechanisms of the response of biological systems at the molecular level. A magnesium alloy implant in the bone of a mammal is corroding more or less fast thus generating $Mg(OH)_2$ and O_2. Thus with the oxidation of corroding Mg oxidative stress is associated as well as un-physiologically high concentrations of Mg ions and other metal ions which may act on the bone cells in proximity to the implant. Therefore two major questions are how bone cells react on the oxidative stress and on the presence of high concentrations of ions released by the Mg alloy implant. Proteomics offer diverse strategies [1], which can yield answers to this question. In general for finding answers to these kinds of questions differential proteomics approaches can be helpful. Usually the protein levels of as many proteins as possible of a control and perturbated system are compared. By the identification of proteins, which concentrations are increased or decreased, and by looking for their functions a mechanism can be proposed, which underlies the response to the perturbation.

METHODS: Bone cells in the absence (control) and in the presence of magnesium implant extract (Mg) are cultivated. For the classical top-down proteomics approach intact cells from both groups are pelleted and subjected to two-dimensional electrophoresis (2DE). 2DE protein patterns are compared, corresponding protein spots, with significant differences in concentration, are excised and digested with trypsin. For bottom-up proteomics (Fig. 1) control cells and Mg cells are lysed and incubated with trypsin. The tryptic peptides of the different approaches are analysed by a system consisting of a liquid-chromatography (LC) coupled to a tandem mass spectrometer (MS/MS). The resulting LC-MS/MS data are processed and used for protein identification by applying a search engine like MASCOT and a protein database such as Swiss-Prot. A relative quantification is achieved either from comparison of the concentrations (density) of the protein spots of the 2DE from Mg cells and control cells or by comparing the areas under the curves of corresponding peaks of their chromatograms of the LC-MS/MS bottom-up analysis. The identified up- or down regulated proteins are analysed by ontology tools which gives suggestions about the protein functions.

RESULTS: The 2DE analysis revealed several dozens of proteins, which concentrations increased or decreased in response to the presence of the magnesium implant extracts.

The bottom-up approach via LC-MS/MS yielded many thousand spectra. By data processing and interpretation many hundreds of proteins were identified and grouped into functional classes.

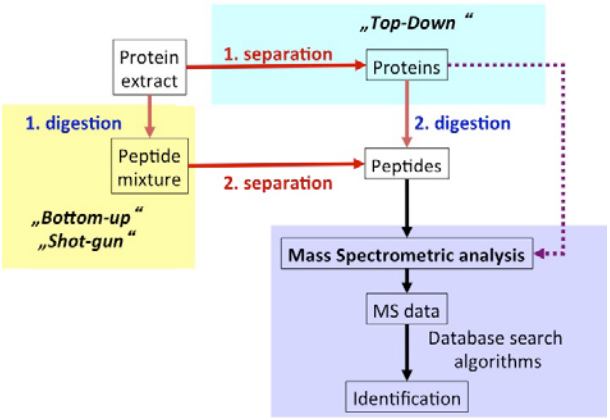

Fig. 1: Main strategies in proteomics.

DISCUSSION & CONCLUSIONS: Proteomics tools are well suited for investigation of system wide responses of cells towards perturbations caused by environmental factors, as demonstrated here by the action of magnesium implant extracts on bone cells.

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ACKNOWLEDGEMENTS: This study was supported by the Helmholtz Association, funding the Virtual Institute MetBioMat.

In vitro and *in vivo* degradation of ultrahigh-pure magnesium

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INTRODUCTION: The degradation performance of magnesium and its alloys is often limited. Too fast degradation interferes with the healing process of the injured tissue and may further cause premature failure of the implant [1]. Therefore, slow and homogeneous degradation is highly desirable. The degradation of Mg is influenced by impurity elements such as Fe, Cu, Ni and Co, and Fe-enriched precipitates accelerate the degradation drastically. They are formed during solidification, but in particular during the course of heat-treatment procedures. This is because the tolerance limit is significantly lower in the near-equilibrium heat-treated state than in the non-equilibrium as-cast condition [2]. For medical applications the reduction of the impurities content is therefore of crucial importance. Here we report on the *in vitro* and *in vivo* degradation of ultrahigh-pure (XHP) Mg in various conditions and compare its performance with high pure (HP) Mg.

METHODS: HP Mg (37 ppm) and three batches of XHP Mg (< 2.2 ppm) were tested in the as-cast, as-annealed and as-extruded states. XHP Mg was produced via distillation in a vacuum apparatus [3]. As-cast rods (HP, XHP) were cast in a vacuum-induction furnace under argon atmosphere. Heat-treatments were performed at 400°C for 48h.As-extruded rods (XHP) were produced at 300°C (ratio 1:69) and measured *in vitro* and *in vivo*.

In vitro degradation tests were performed by immersing polished discs in simulated body fluid (SBF) at 37°C and pH 7.45±0.06. The degradation rate was evaluated according to their hydrogen (H_2) evolution. The solution was buffered with carbon dioxide (CO_2). The newly designed setup made of burettes, funnels and a frit allows for a correction of the atmospheric pressure and gas dissolution. This assembly allows mimicking the *in vivo* situation very precisely [4, 5].

In vivo experiments were conducted in six male Sprague-Dawley rats. XHP Mg pins of 1.6 mm in diameter and 8 mm in length were implanted into the femur of the rodents. Micro CT scans were performed at 1, 4, 8 and 12 weeks after pin implantation.

RESULTS: *In vitro* experiments of as-cast and heat-treated HP and XHP Mg show clearly that HP Mg corrodes faster than XHP Mg. Heat-treated HP samples release higher H_2 amounts than their counterparts, and annealed and as-extruded XHP Mg exhibit similar hydrogen evolution than as-cast XHP Mg. From the H_2 evolution, the annual degradation rate was calculated to be P_H = 10 ± 3 µm/y for XHP Mg, 28 ± 2 µm/y for HP Mg and 39 ± 3 µm/y for HP400 Mg. The average *in vivo* degradation rate was determined from the volume loss during the 3-month implantation period. A value of P_{IV} = 13 ± 3 µm/y was evaluated, which is in very good agreement with the *in vitro* rate.

DISCUSSION & CONCLUSIONS: XHP Mg samples released less H_2 due to the extremely low impurity level, which generated no cathodically acting precipitates. In contrast, annealed HP discs corroded faster than their as-cast counterparts. Thermodynamic calculations indeed confirmed the experimental observation of a higher volume content of Fe-containing BCC phases in as-annealed HP-Mg compared to the as-cast condition. The impurity level in XHP Mg was below the tolerance limit and thus thermo(mechanical) treatments did not generate accelerated degradation compared to the as-cast state.

In addition, the very good agreement of the *in vivo* results with the *in vitro* data of XHP Mg confirm the accuracy and reliability of the newly designed corrosion setup.

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Are biodegradable Mg-based implant materials suitable for the use in osteosynthesis systems?

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INTRODUCTION: Biodegradable osteosynthesis systems for temporary use in fracture repair are desirable to avoid a second surgery for implant removal. In previous studies, the implant material LAE442 showed a good biocompatibility combined with slow degradation rate and high mechanical strength [1]. Therefore it was the aim of the present studies to examine LAE442-based osteosynthesis-systems (plates and intramedullary nails) in *in vivo* experiments.

METHODS: Extruded LAE442 rods (Ø 10mm) were used as bulk material to manufacture plate screw-systems (plates: 31 mm x 6.4 mm x 1.8 mm and 2.8 mm in hole Ø; screws Ø 2.4 mm, 6.1mm length) and intramedullary nails with interlocking screws (nails: Ø 9 mm, 130 mm length; screws: Ø 3.5 mm, length variable). Plates were fixed monocortically at the medial aspect of the tibia of NZW rabbits. Nailing systems were implanted into the tibiae of sheep via a surgical approach in the knee joint and interlocked with four 90° shifted screws. To examine the functionality, a fracture model was used (pilot study, identical implantation procedure and osteotomy. All surgical procedures were performed under general anaesthesia with an intra- and postoperative antibiotic and analgesic treatment for at least 10 days. Besides postoperative clinical examinations, radiographic and (µ-) computed evaluations were performed as well as histologic analysis of the explanted bone-implant-compounds after euthanasia. The animal experiments were authorized according to the German Animal Welfare Act (no. 33.12-42502-04-11/0327; no. 33.12.-42502-04-10/0106).

RESULTS: Plate-screw-systems showed undesired gas formation with massive bone reactions and new bone formation around the implant as well as osteolysis processes (Fig. 1A). In contrast, in intramedullary nailing systems an adequate biocompatibility, moderate gas formation and moderate increased bone reactions especially around the distal interlocking screws were observed (Fig. 1B). In the fracture model, an insufficient mechanical stability caused implant failure two days postoperatively (Fig. 1C).

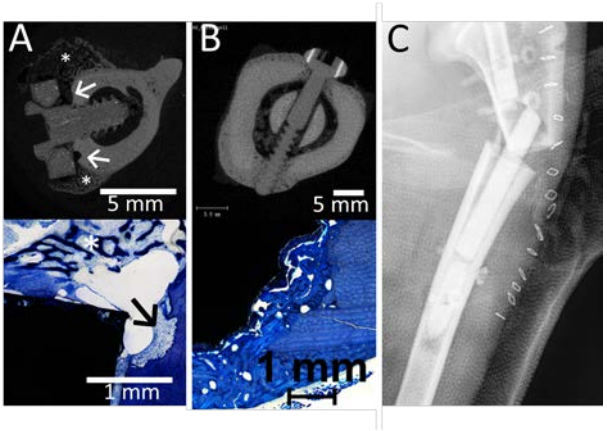

Fig. 1: µ-CT and histological pictures of screw-plate-systems (A) with massive bone reactions (*) and osteolysis (↑); intramedullary nail systems in non-osteotomized tibiae (B); radiographic picture of failed implant in osteotomized sheep model (C).

DISCUSSION & CONCLUSIONS: The use of Mg-based osteosynthesis-systems in high loaded bones for fracture repair has to be considered as critical. Beneath high amounts of corrosion products and clinical problems with occurring gas as well as bone reactions, mechanical stability seems to be insufficient even in a magnesium alloy with a comparably high mechanical strength [1]. Consequently, Mg-based materials should be preferably used for implant materials with smaller geometries and lower load.

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Rational approach for evaluating Mg alloys for their biocompatibility

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INTRODUCTION: Currently there is immense interest in magnesium (Mg) alloys for their potential applications in biodegradable orthopaedic devices. In the evaluation of biocompatibility of these material it is important to understand the effects of corrosion byproducts on the physiological environment where these are implanted. Most investigations step-up to *in vivo* intraosseous studies immediately after *in vitro* biocompatibility evaluation. The relatively high degradable nature of Mg frequently exhibit false cytotoxic attributes in static mono-layer cell culture environments due to osmotic shock and rapid pH changes. This can result in the unnecessary elimination of materials with clinical potential.

METHODS: We propose a combination of relevant cell culture and small animal soft tissue implantation as an intermediate step prior to large animal intraosseous investigations.

RESULTS: The viability of fibroblast like L929 and osteoblast like SaOS-2 cells exposed to Mg alloys were compared to those seen with the intramuscular implantation of the same materials in Lewis rats.

DISCUSSION & CONCLUSIONS: This approach was successful in the identification of materials with appropriate biocompatible/ degradable properties suitable for proceeding to intraosseous investigations.

Study on *in vitro* biocompatibility and macrophage phagocytosis of $Mg_{17}Al_{12}$ in Mg-Al-Zn alloys

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INTRODUCTION: Mg-Al-Zn series alloys are widely studied as potential biodegradable implant materials for orthopedics and vascular stent applications due to their excellent mechanical properties and corrosion resistance[1]. $Mg_{17}Al_{12}$ as a commonly existing second phase in Mg-Al-Zn alloys was proved of lower degradation rate than the Mg matrix due to its higher corrosion potential [2], therefore probably leaching into the physiological environment along with the degradation of alloys and leading to a host response such as inflammation. In the present study, the *in vitro* biocompatibility and a possible metabolic behaviour (macrophage phagocytosis) of $Mg_{17}Al_{12}$were investigated.

METHODS: A single $Mg_{17}Al_{12}$ compound was fabricated to simulate the degradation behavior of the separated second phase after the thorough degradation of the Mg matrix. Tests of hemolysis, cytotoxicity, cell proliferation and cell adhesion were adopted to investigate the biocompatibility of the $Mg_{17}Al_{12}$ second phase. In addition, $Mg_{17}Al_{12}$ particles were prepared to simulate the real second phase in the *in vivo* environment and to see the cellular response in macrophages to the $Mg_{17}Al_{12}$ particle.

RESULTS: The hemolysis rate of $Mg_{17}Al_{12}$ (0.57%) and pure Mg as control (1.72%) were both below 5%, meaning that $Mg_{17}Al_{12}$would not lead to severe hemolysis. The cytotoxicity test indicated that $Mg_{17}Al_{12}$ showed no toxicity to L929 cells, MC3T3-E1 cells and BMSCs, meanwhile the cell viabilities in the extract of $Mg_{17}Al_{12}$ were all higher than those in the pure Mg extract. The cell proliferation test showed that cells cultured in the extract of $Mg_{17}Al_{12}$ led to a significant increase of O.D. value with the incubation time. The cell adhesion observed by DAPI staining shown in Fig.1 indicated that the amount of cells on the $Mg_{17}Al_{12}$ surface was obviously increased along with the incubation time. The macrophage phagocytosis of $Mg_{17}Al_{12}$ particles were illustrated by both SEM and TEM images, as shown in Fig.2, showing that the RAW264.7 macrophage exhibited good survival and stretched out of the filopodia. The $Mg_{17}Al_{12}$ particles were taken up by the macrophage (Fig.2a). The shapes of the organelles were complete and the $Mg_{17}Al_{12}$ particles were digested by the lysosomal enzyme in the lysosome (Fig.2b).

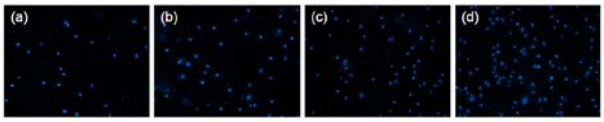

Fig. 1: Fluorescence images of MC3T3-E1adhesion on $Mg_{17}Al_{12}$ stained with DAPI after30min (a), 60min (b), 90min (c) and 120min (d) of incubations.

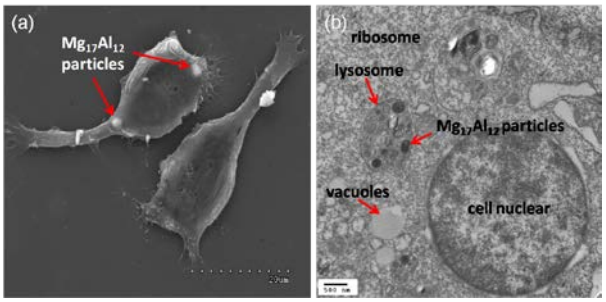

Fig. 2: Typical SEM image (a)and TEM image (b) of RAW264.7 phagocytizing $Mg_{17}Al_{12}$ particles after co-culture for 24h.

DISCUSSION & CONCLUSIONS: $Mg_{17}Al_{12}$ is a typical second phase in Mg–Al–Zn alloys, which should be paid more attention on degradation. No hemolysis of $Mg_{17}Al_{12}$ was found and the blood compatibility of $Mg_{17}Al_{12}$ was much better than that of pure Mg. Good cyto-compatibilities of $Mg_{17}Al_{12}$ were shown when co-cultured with different types of cells. The macrophage phagocytosis of $Mg_{17}Al_{12}$ particles could happen and then were digested by the lysosomal enzyme.

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Effect of Mg-2Sr alloys on Fracture Healing in Osteoporotic Rats, via SMAD1/5/8 Signaling

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INTRODUCTION: Due to low bone mass and deteriorated bone microarchitecture, osteoporotic fracture patients have a high risk of delayed fracture healing and bone non-union. A Mg-2Sr alloy was developed as a new type of biodegradable metal, with optimal strength, corrosion resistance, and mechanical properties, as well as good biocompatibility. We investigated whether Mg-2Sr alloys could stimulate osteoblast differentiation and mineralization from ovariectomized (OVX) rats, by mimicking postmenopausal osteoporosis, and whether Mg-2Sr alloys facilitated fracture healing in OVX rats when implanted after an osteoporotic fracture.

METHODS: We mimicked the *in vivo* process of Mg-2Sr degradation and obtained Mg-2Sr degradation products (Mg-2Sr-DP) by immersing Mg-2Sr in culture medium. Bone marrow cells were obtained from OVX rats. We examined the *in vitro* cytotoxicity of Mg-2Sr-DP using cell count kit-8, flow cytometry, and growth cycle assays. The effect of Mg-2Sr-DP on osteoblast proliferation, differentiation, and mineralization were examined using alkaline phosphatase and alizarin red staining. *In vivo*, 3 months after ovariectomy, 80 female Sprague–Dawley rats underwent bilateral osteotomy of their femur diaphyses, which were subsequently fixed with intramedullary wires. The animals were divided into three groups: OVX+pure Ti, OVX+pure Mg, and OVX+Mg-2Sr. Bone callus quality was evaluated at 4 and 8 weeks, post-fracture. Finally, molecular techniques were used to identify the potential mechanisms through which Mg-2Sr-DP stimulated osteoblast proliferation and differentiation, *in vitro* and *in vivo*.

RESULTS: *In vitro*, Mg-2Sr-DP promoted OVX-derived osteoblast proliferation and differentiation. This was further supported by the observation of a significant increase in the expression of osteoblast-specific markers, including runt-related transcription factor-2, osterix, bone sialoprotein, osteocalcin, and type I collagen. *In vivo*, compared with the pure Ti and pure Mg groups, Mg-2Sr-treated animals demonstrated significantly increased bone formation, bone mineral density, biomechanical strength, and improved microstructural properties of the callus. The ultimate load and total bone volume of the callus was increased significantly at 4 and 8 weeks, post-fracture. Mg-2Sr treatment also promoted healing, with increased osteogenesis at 4 weeks; in addition, more mature woven or lamellar bone was observed at 8 weeks across the fracture gap. Further molecular analysis revealed that Mg-2Sr-DP inhibited I-κB degradation, abrogated NF-κB nucleus translocation, and subsequently advanced the DNA binding of pSMAD1/5/8, thereby stimulating osteoblast differentiation. Furthermore, tumor necrosis factor (TNF)-α severely impaired BMP2-induced osteoblast differentiation; this inhibition could be greatly attenuated by Mg-2Sr-DP. Thus, we propose that Mg-2Sr-DP acts by inhibiting TNF-α-induced NF-κB signaling to promote osteoblast differentiation, thus further facilitating fracture healing in rats exhibiting a condition similar to postmenopausal osteoporosis.

DISCUSSION: This study suggests that intramedullary Mg-2Sr treated wires can promote femur diaphyseal fracture healing in OVX rats.

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In vitro study on the corrosion protective effect of cells adhesion on biodegradable Mg-Nd-Zn-Zr alloy

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INTRODUCTION: In biodegradable metals, the interaction between magnesium alloys and cells need to be clarified. Many studies have focused on the cells response to magnesium alloys, while few papers report how the cells affect the corrosion behavior [1-2]. This study aims to verify the protective effect of human osteoblasts on the corrosion of Mg-Nd-Zn-Zr alloy (JDBM).

METHODS: JDBM samples of Ø8×2 mm were cut from extruded bar, and then polished to 5000 grade, etched and sterilized. 5000 human osteoblasts were seeded on each sample in 24-well plate. After 1d, 3d culture, the samples were fixed in 2.5% glytaradehyde over night and subsequently in 1% osmium tetroxide for 0.5 h. After gradient dehydration in 20%, 40%, 60%, 80%, 95%, 100% isopropanol, samples were dried by critical point drying method. Then the samples were examined using scanning electron microscope (SEM, Zeiss DSM 982 Gemini) and focused ion beam SEM (FIB-SEM, Zeiss Auriga).

RESULTS & DISCUSSION: After 3 days culture, the osteoblasts are well spread, and attach tightly on the surfaces of JDBM samples as shown in Fig. 1a. A corrosion layer formed on the JDBM surface, which is uniform and dense, with net-like structures. Notably, the corrosion layer is not completely flat. The layer at the cells and nearby spots seems lower and denser than the other place. Therefore, we assume that the cells could affect the corrosion behavior of JDBM alloy in two ways: 1. the cells themselves could provide the protection, probably by producing extra cellular matrix (ECM) which was absorbed onto the alloy surface. This may result in a lower corrosion rate and thinner corrosion layer. 2. The cells make the corrosion layer denser by active secretion of Ca, which is then precipitating in the corrosion layer underneath the cell.

To confirm this result, the 3days-culture samples were observed under FIB-SEM. Focused ion beam was used to dig a long pit through a cell, by which the cross section of corrosion layer was exposed, as shown in Fig. 1b. We can find that at the cell position, the corrosion layer is more uniform, with a thickness of about 5 µm, while at other position the thickness varies from 3 to 8 µm. The average thickness at no-cell position is larger than that at cell position. This result suggests the cell could protect the alloy substrate.

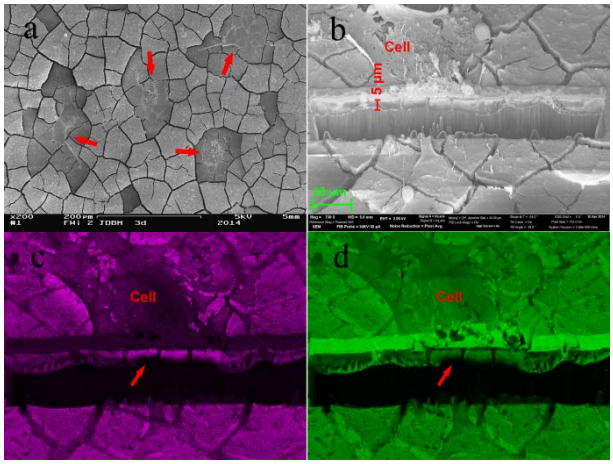

Fig. 1a: SEM image of 3days-culture sample. The red arrows point to osteoblasts. Fig. 1b: FIB-SEM image of the cross section of the corrosion layer. Fig. 1c: Mapping of Ca element. Fig. 1d: Mapping of P element.

Fig. 1c and Fig. 1d are energy dispersive x-ray spectroscopy (EDS) mappings of Ca and P elements. It is found that the element distributions in the corrosion layer were influenced by the cell. Ca and P tend to concentrate at the upper part of the layer close to the cell, while at the sublayer the concentrations are much lower than normal. This is probably due to the active secretion of Ca by cell. The Ca precipitation forms a dense layer underneath the cell, which stops Ca diffusion into the lower part of the corrosion layer.

CONCLUSIONS: Osteoblasts adhesion on the surface of JDBM is altering the corrosion layer either by ECM adsorption and/or local changes in the elemental composition of the corrosion layer.

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Effect of magnesium extract on osteoblastic progenitor cells differentiation

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INTRODUCTION: Even if already investigated during the first half of the last century [1], the interest of using magnesium-based implant material decline until recently. The unique properties of Mg and its alloys to combine metal related mechanical properties, biocompatibility, and biodegradability (up-to now restricted mainly to polymers and calcium-phosphates) justify this renewal of attention. It is also for century suspected and observed that Mg-based implants induce bone formation in musculoskeletal implantation sites [2]. However, except the fact it is also known that Mg is increasing general cell metabolism (especially because of its interdependence with ATP), no broad but still comprehensive researches have been led to understand the mechanical mechanisms implied in this phenomenon. In an (*in-vitro*) attempt to reveal the underlying mechanisms of the possible osteoinductivity of magnesium, nonhematopoietic multipotent foetal progenitor cells *i.e.*, HUCPV or human umbilical cord perivascular, were employed.

METHODS: HUCPV were cultured for up to three weeks with or without osteoblastic differentiating media and with or without magnesium extract (pure Mg-extract; concentration of about 5 mM). Every week, effect of magnesium was studied on gene level but also on protein level. The first method employed was real-time polymerase chain reaction. In order to find suitable markers, not only classical bone markers (*e.g.*, osteocalcin (OC), collagen 1A1 (COL1A1), and runt-related transcription factor 2 (RUNX2)) were studied but also genes involved in (*e.g.*) osmotic stress, metal channels, and adhesion molecules were examined. More than 80 genes were selected and studied. In a second step, mineralisation (*via* alizarin-s staining and alkaline phosphatase (ALP) activity) and different proteins were targeted (*via* Elisa tests).

RESULTS: Addition of pure Mg-extract alone modified expression of several genes already on the first week. For example, up- and down regulation of bone morphogenetic proteins (*e.g.*, BMP 2, 4, and 6) was observed over the 3 weeks. ALP activity was enhanced in cells cultured with Mg-extract and differentiation media. The COL1A1 protein expression was stable in the first week (1W) between all conditions, while a synergistic effect was observable (Mg-extract and differentiation media) on weeks 2 and 3 (2W and 3W, respectively; see *Fig. 1*).

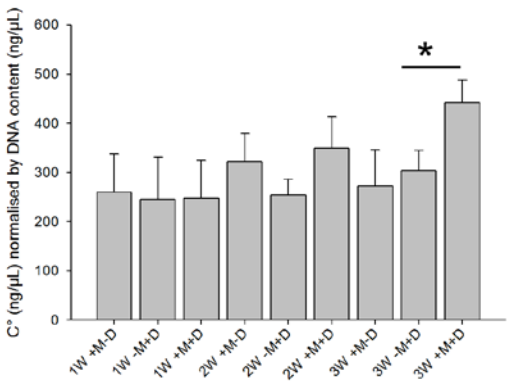

Fig. 1: COL1A1 protein expression normalized by DNA content.

DISCUSSION & CONCLUSIONS: The RT-PCR results highlighted several suitable markers. Taking the results all together, it was measured that cells were differentiating and that Mg-extract was enhancing the effect of the inducing differentiation media. Most of the time, a synergistic effect was observable. Mg not only increased osteoblast differentiation and activity but also increased bone remodelling (*e.g.*, osteoblast-dependant osteoclast induction).

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Endothelial growth on fluoride and collagen coated magnesium alloys

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INTRODUCTION: Magnesium (Mg) alloys are promising scaffolds for the next generation of cardiovascular stents because of their better biocompatibility and biodegradation compared to traditional metals. However, the high degradation rate of current Mg-based alloy heavily compromises its performance in cardiovascular application. Hydrofluoric acid (HF) treatment and collagen coating were used in this research to improve the endothelialization (a healing process after stent implantation) of two rare earth-based Mg alloys. The goal of this study was to evaluate the fluoride coated material and to compare the results to collagen coated and uncoated Mg-RE alloys for endothelialization.

METHODS: Electrochemical corrosion test was used to measure the corrosion rate of 3 Mg materials. Scanning electron microscopy (SEM) was used to characterize the surface morphology of collagen coated and hydrofluoric acid treated Mg alloys. EDS was used to measure the thickness of fluoride coating. Primary human coronary artery endothelial cells (HCAECs) were cultured on different materials directly. Cell viability was tested by LIVE/DEAD assay.

RESULTS: Collagen showed different morphology structure on 3 materials. Collagen sheet mixed with the long collagen fiber covered the entire surface of Mg-RE2 alloy. The electrolytes released from the degradation process could affect the assembly of collagen on Mg alloys. SEM images demonstrated that a nanoporous film structure of fluoride with thickness of ~20 μm was formed on the Mg material surface, which improved the corrosion resistance. HCAECs had much better attachment, spreading, growth and proliferation (the process of endothelialization) on HF-treated Mg materials compared to bare- or collagen-coated ones.

DISCUSSION & CONCLUSIONS: Collagen and HF coatings were successfully prepared on rare earth-based Mg alloys intended for cardiovascular applications. HF treatment could modify Mg surface into a nanoporous layer. The size and structure of modified layer are dependent on the chemical composition of the alloy. In comparison to the non-coated Mg samples, both coatings significantly decreased the degradation rate with HF having the lowest rate of degradation. As a result, HF treated material demonstrated better endothelial cell attachment and proliferation than bare Mg or the same material coated with collagen. Such better endothelialization on HF coated materials could result from a combined effect including slower degradation, less pH change, less hydrogen gas release and nanoporous structures on the surface for easier cell attachment. These findings indicate the HF coating prepared in the current study is promising for controlling the biodegradation and improving the cytocompatibility of rare earth-based Mg alloys. However, more future cell and animal studies are needed in order to translate such Mg implants to clinical applications.

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Magnesium alloys in implantology: the effects of high concentrations of magnesium on human osteoblasts

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INTRODUCTION: Due to their mechanical properties, their biodegradability and biocompability, magnesium (Mg) alloys are good candidates for biomedical implantable devices, especially in the area of orthopedics [1]. Indeed, Mg-based implants can provide temporary structural support, thus preventing complications associated with the long-term presence of implants or the risks of secondary surgery. Even though the corrosion rate of these alloys can be controlled, their degradation leads to a high Mg concentration around the implant and this event has been shown to influence the surrounding bone structure, also by interfering with the activity of bone cells. We pointed our attention to the effects of increased extracellular Mg on human pre-osteoblast differentiation and in osteoblast activity.

METHODS: Physiologic Mg levels are approximately 1.0 mM. Human primary osteoblasts and pre-osteoblasts were exposed to 1.0, 3.0 and 5.0 mM Mg (Mg chloride and sulfate). Cells were counted using a cell counter. To induce differentiation, pre-osteoblasts were exposed to an osteogenic medium (OM) containing vitamin D and L-ascorbate-2-phosphate and alkaline phosphatase activity was measured at different times. To study osteoblast activity, calcium deposition by Alizarin Red staining was evaluated. Intracellular Mg was measured using a spectrofluorimetric assay and the Mg transporters TRPM6 and 7 were detected by western blot.

RESULTS: Initially, we found that the concentrations of intracellular Mg remained unchanged after culture in 1.0, 3.0 or 5.0 mM Mg. Accordingly, it is noteworthy that high extracellular Mg did not affect the total amounts of the cation transporters TRPM6 and 7, which are responsible for intracellular Mg homeostasis.

Then we showed that culture in 5.0 mM Mg stimulates the growth of osteoblasts and pre-osteoblasts.

High extracellular Mg, however, inhibited ALP activity in pre-osteoblasts exposed to OM up to 20 days. In osteoblasts, culture in high Mg reduced calcium deposition. $MgSO_4$ and $MgCl_2$ exerted the same effects at the same concentrations.

DISCUSSION & CONCLUSIONS: Differently from other cell types, high extracellular Mg does not impact on the amounts of Mg transporters and on Mg intracellular homeostasis [2]. While high Mg enhances cell growth, it retards pre-osteoblast differentiation and it impairs osteoblast activity. On the contrary, high Mg does not affect the differentiation of endothelial cells and pre-adipocytes, thus demonstrating that the differentiation of different cell types is differently regulated in response to extracellular Mg.

Since TRPMs conduct other essential metals such as calcium, manganese, zinc and copper, which are cofactors for many essential cellular enzymes and/or transcription factors, we propose that high extracellular Mg might alter the intracellular cation balance, thus interfering with various cellular functions. However more experiments are needed to elucidate the role of Mg and its transporters in driving bone cell differentiation and function, especially considering the potential large use of Mg alloys in biomedicine.

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In vitro biocompatibility of novel magnesium-rare earth based stent materials

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INTRODUCTION: As the next generation of biomaterials, magnesium (Mg)-based alloys are promising candidates for cardiovascular implants including pacemakers, coronary stents and heart valves. Their advantages include relative abundance, similar density to bone, strength, elasticity, stress shielding, biodegradable, and involvement in metabolism and other biological activities. For biomaterials, biocompatibility is as important as mechanical properties, but such biocompatibility analyses on Mg and its alloys are not yet fully addressed. Thus, it is critical for biocompatibility of Mg and its alloys to be completely explored before any pre-clinical/clinical applications. Novel Mg alloys have been manufactured and characterized, and in vitro biocompatibility assessments of these materials intended for cardiovascular applications have been conducted.

METHODS: Four magnesium-rare earth alloys intended for stent application were manufactured, and their mechanical and corrosion properties were evaluated. In vitro biocompatibility analyses including hemolysis, platelet adhesion, aortic endothelial cell viability, and endothelialization were systematically performed.

RESULTS: In this study, we evaluated MgZnCaY-1RE, MgZnCaY-2RE, MgYZr-1RE, and MgZnYZr-1RE alloys for cardiovascular stents applications regarding to their mechanical strength, corrosion resistance, hemolysis, platelet adhesion/activation, and endothelial biocompatibility. The mechanical properties of all alloys were significantly improved. Potentiodynamic polarization showed that the corrosion resistance of four alloys was at least 3-10 times higher than that of pure Mg control. Hemolysis test revealed that all the materials were non-hemolytic while little to moderate platelet adhesion was found on all materials surface. No significant cytotoxicity was observed in human aorta endothelial cells cultured with magnesium alloy extract solution for up to seven days. Direct endothelialization test showed that all the alloys possess significantly better capability to sustain endothelial cell attachment and growth. The results demonstrated the promising potential of these alloys for stent material applications in the future.

DISCUSSION & CONCLUSIONS: Four novel Mg-RE alloys were successfully fabricated and tested for the feasibility as vascular stent materials with pure Mg as control. The microstructure, mechanical properties, corrosion resistance, hemocompatibility, and endothelial cytocompatbility were systematically evaluated. The microstructures of all four materials were significantly refined after the addition of RE elements. All the mechanical properties were remarkably improved. The corrosion rate was reduced at least 3 to 10 times than that of pure Mg control. Surprisingly, the addition of RE elements in Mg didn't bring any significant deleterious effects on hemolysis and the platelet adhesion, instead reduced platelet adhesion and activation were observed on all RE alloys. Indirect MTT test revealed that there was no significant difference between cell viability in RE alloys and pure Mg control in the static culture system. Direct endothelialization test showed that RE alloying could significantly improve cell attachment and spreading on the alloy surfaces. Taken together, these Mg-RE alloys are demonstrated with promising features as stent materials, and future in vivo studies are needed to fully assess their potential for cardiovascular stent applications.

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Proteomics study of mouse fibroblasts exposed to biodegradable pure magnesium

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INTRODUCTION: Magnesium and its alloys are interesting candidates for orthopaedic and cardiovascular biomaterials due to their biodegradability. However, most in vitro biocompatibility studies of biomedical used magnesium still rest on cellular levels. How the materials act on cells in molecular level is less known. This proteomics study of mouse fibroblasts exposed to biodegradable pure magnesium could provide a more comprehensive understanding the interact mechanism between cells and degradable materials.

METHODS: In this work, we established the protein expression profile change of mouse fibroblasts L929 exposed to as-extruded pure Mg in DMEM extract and DMEM for 8, 24, 48 h respectivly by isobaric tag for relative and absolute quantitation (iTRAQ)-coupled two dimensional liquid chromatography-tandem mass spectrometry (2D LC-MS/MS) approach and bioinformatics analysis to reveal the molecular mechanisms of the interaction between magnesium and cells. These results were confirmed by quantitative RT-PCR. WST-8 cell proliferation assay and flow cytometry were used to evaluate the cellular response of L929 cells to pure Mg.

RESULTS: The magnesium ion concentration has remarkably increased in from 21.7μg/ml to 92μg/ml, on the contrary, the calcium ion concentration drops from 82.8μg/ml to 60.5μg/ml in DMEM control groups and the Mg extract respectively. After exposed in the Mg extract for 8, 24 and 48h, the viability of L929 cells is at the same lever to the negative control and proliferation index (PI) of L929 is significantly higher in Mg extract than that of control group.

Compared with the control groups, L929 cells exposed to pure Mg extract were associated with the 205, 282 and 217 differently regulated proteins for 8, 24 and 48h respectively. 25 changed expressed proteins involved in all the three time points were screened from all the changed data. Among the 25 proteins, 8 proteins were down-regulated and 4 proteins were up-regulated all through the incubation time, while the expression of the rest proteins changed at different exposed time. Gene ontology (GO) annotation of the differentially expressed proteins showed that the L929 cells which responded to pure Mg covered a broad range of functional groups including cellular biological process, molecular function, and cellular component. KEGG pathway analysis illustrated that the differently expressed proteins were mainly involved in cardiac muscle contraction, focal adhesion oxidative phosphorylation processing.

DISCUSSION & CONCLUSIONS: Through proteomic approaches, pure Mg did not significantly influence the expression profile of L929 fibroblasts, though it could induce a cooperative response involving a wide panel of proteins and biological pathways. Cell could auto-regulated itself when exposed to a different environment. Some pathways such as focal adhesion will be studied in detail in the further work since it has been specially influenced. The protein profile established in our study is an effective approach to clarify molecular mechanisms of cell-biomaterial interaction

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In vivo characterization of brushite coated Mg–Nd–Zn–Zr alloy for mandible bone repairing applications

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INTRODUCTION: Mg-Nd-Zn-Zr alloy has been proven to adopt nanophasic biodegradation mechanism to enhance durability and biocompatibility. In vitro characterization of Mg-Nd-Zn-Zr alloy and brushite coated Mg-Nd-Zn-Zr alloy have been discussed before [1], neither of them has showed biotoxicity. In this work, In vivo characterization of brushite coated Mg-Nd-Zn-Zr alloy used for mandible bone repairing has been evaluated to establish its potentials for degradable craniofacial bone repair material.

METHODS: A total of 10 bushite coated Mg-Nd-Zn-Zr screws (4.6 mm in thread length) were implanted to fix the intentional defects in mandible bone of 10 healthy adult New Zealand rabbits. Samples were collected 1 and 4 months post implantation. Synchrotron X-ray microtomography was performed to determine biodegradation characterization of the implants. Histological analysis was carried out with samples embedded in polymethylmethacrylate (PMMA), sliced and stained with Van Gieson's picric acid-fuchsin.

RESULTS: Three-dimensional imagines of screws 1 and 4 months post-operation are presented in Fig.1. Volume loss and in vivo degradation rate are calculated and demonstrated in Table 1. Brushite coated Mg-Nd-Zn-Zr screw experienced a low rate of degradation in the first 4 months of implantation due to ceramic coating.

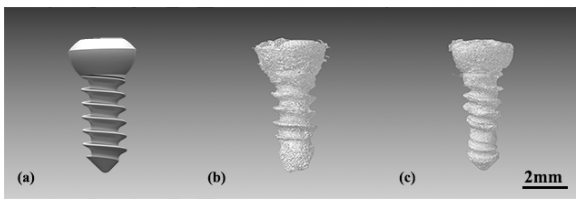

Fig. 1: Drawing of a screw (a) and 3-D reconstruction images of screws after 1 month (b) and 4 months (c) of implantation.

Fig. 2 shows histological analysis of interface between coated metal screws and tissues. Foreign body reaction was weak and no inflammatory cells or acute inflammation processes were observed in any samples. Amount of osteoid and osteoblasts were detected adjacent to the metal screw after 1-month implantation. Bone trabecular was arranged in good order. Osteocyte and osseous lamella were evidenced in 4-months post operation samples, which suggested osteogenesis. Intentional bone defect had recovered by 4 months.

Table 1. Volume loss and in vivo degradation rate of brushite coated Mg-Nd-Zn-Zr screws.

	Volume (mm^3)	Volume Percentage	Degradation Rate(mm/year)
1 month	11.88	81.21%	0.54
4 months	10.72	73.28%	0.19

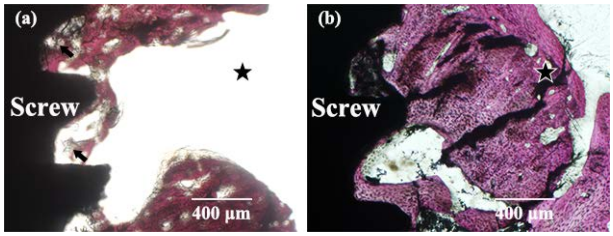

Fig. 2: New bone formation and intentional bone defect recovery 1month (a) and 4 months (b) post operation. (arrow-osteoid; asterisk-intentional defect site)

DISCUSSION & CONCLUSIONS: Brushite coated Mg-Nd-Zn-Zr alloy screws have manifested suitable in vivo biodegradation rate as mandible bone repairing material. Brushite coating had significantly enhanced corrosion resistance at the incipient stage of implantation. Mg-Nd-Zn-Zr alloy had exhibited to facilitate osteanagenesis and osteogenesis, and it had been confirmed to be a promising option for mandible bone repairing.

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Osseointegration of resorbable magnesium screws – A SRμCT Study

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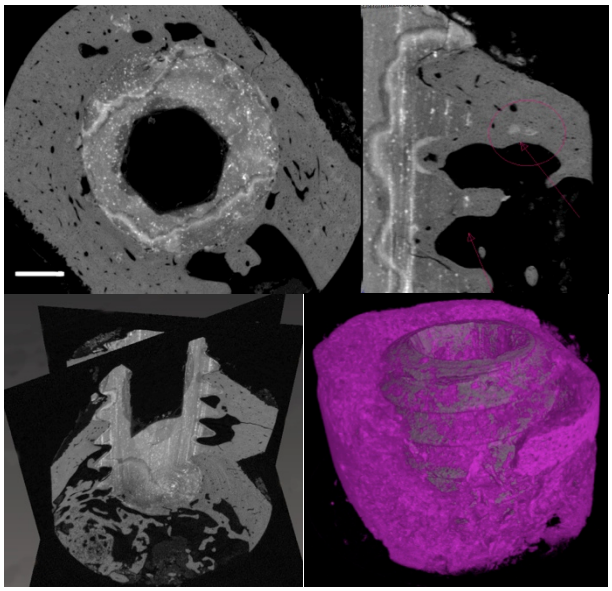
INTRODUCTION: The development of resorbable osteofixation materials that degrade upon substitution by regenerated tissue is highly desirable in orthopaedics. Magnesium is promising as implantable material, because of its biocompatibility, osteoconductivity and biodegradation under physiological conditions [1]. Through the selection of alloying elements, the mechanical properties and corrosion behaviour of magnesium can be modulated for application in load-bearing situations. The aim of our research was to investigate the bone integration and the corrosion process of Al-free Mg-alloys *in vivo*. Our hypothesis was that Mg-based implants stimulate bone growth.

METHODS: Mini-screws of two different Mg-alloys, Mg10Gd and Mg-Y-RE (WE43) were manufactured at HZG. The cytocompatibility of the selected alloys was formerly tested and validated in vitro [2, 3]. The mini-screws were implanted in rats after ethical approval. After 1 and 3 months of healing, cylindrical bone-implant blocks were retrieved. Samples were imaged at the P05 Imaging Beamline (IBL) operated by HZG at PETRA III – DESY (Hamburg). We used monochromatic X-rays at 25 keV to take 900 projections and a field of view of 7mm x 1.8 mm, which resulted in 5X magnification with a resolution of ~2.5 μm. 3D data sets were computed using filtered back projection algorithms.

RESULTS: The inserted implants healed without any observable adverse effect. On the basis of tomographic data, we were able to compute three-dimensional renderings of dvrscrews and bone with high contrast-to-noise ratios. A qualitative evaluation of the data revealed inhomogeneous surface corrosion of the screws, which maintained their original shape within the study period. New bone formation was observed in all of our samples. We found a considerable increase of implant-bone contact sites with progressing healing time. A quantitative analysis of the tomographic data indicated spatial differences in bone density. In proximity of the implant, newly formed bone matured and became dense after 3 months.



Top: Horizontal (left) and vertical (right) sections of a screw after 3 months of healing. Fragments of implants, completely integrated in the bone, are visible. Bar 0.25 mm. Bottom: Orthogonal cut planes (left) and volume rendering (right), showing an implant (gray) into the bone (purple).

DISCUSSION & CONCLUSIONS: The SRμCT showed osseointegration of Mg10Gd and WE43. Although the spatial resolution was not sufficient to fully elucidate the alloys microstructure, we observed the distribution of the high absorbing regions in the materials, possibly intermetallic phases and Y or RE oxides. The corrosion of the alloys was slow. Biocompatibility of the tested materials was confirmed by bone growth in intimate contact with the implants.

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Effect of Mg particles on MC3T3-E1 and J774 cellular cycle. Influence of fluoride treatment

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INTRODUCTION: Mg-based materials are promising for orthopedic, dental, and cardiovascular applications [1]. Magnesium has a high degradation rate that causes the release of microscopic debris particles (MgP) and metal ions upon implanting. Protective treatments involving the generation of magnesium fluoride conversion coatings (Mg-F-C) have been proposed to minimize its high reactivity [2]. The aim of this work was to evaluate the possible cytotoxic effects of MgP and the effect of a fluoride treatment on the cytotoxicity of these particles (MgP02) on macrophage (J774) and osteoblast (MC3T3-E1) cell lines.

METHODS: MgP (99.8%, 325 mesh, 58.9 µm ± 20.7 µm.) were supplied by Alfa Aesar and were used to simulate Mg debris. Mg-F-C were generated by immersion of MgP into 0.2M KF (MgP02) for 1h at room temperature. After the immersion period, KF-treated MgP were suspended in DMEM and subsequently added to cell cultures at a final concentration of 1 mg/ml. The J774 and the MC3T3-E1 cell lines both from *Mus musculus* origin were used to test the immune and the osteoblast cell response to the particles. The effect of MgP and MgP02 and their degradation products on cell cycle and cellular viability were analyzed by flow cytometry (FC) analysis (XL Flow Cytometer, Beckman Coulter Corp) using propidium iodide. The presence of fluoride layer on the MgP02 was detected by mass spectroscopy (Maldi-TOF) analysis.

RESULTS: Cellular viability rate and cell percentages included in the different cell cycle phases were evaluated in absence and in presence of 1 mg/ml of MgP and MgP02 by FC (Table 1). Machophages were more affected than MC3T3-E1 cells by the two types of particles assayed, specially by those treated with fluoride. J774 viability decreased considerably in presence of particles as a reduction of 24.4 % and 45.5 % was observed in presence of MgP and MgP02 respectively. The percentage of $SubG_1$ phase, which is apoptosis-related, increased 5.1% and 11.6% in presence of MgP and MgP02, respectively while the synthesis phase (S) decreased 18.3% and 12.4% with respect to control (in absence of particles). Slight changes in viability of MC3T3-E1 cells and no change in cell cycle phase were observed by MgP and MgP02

Table I. Viability of MC3T3-E1 and J774 cells included in different cell cycle phases.

	MC3T3-E1		
Surface	Viability (%)	$SubG_1$ (%)	S (%)
No particles	94.9	3.5	13.9
MgP	74.5	4.4	13.2
MgP02	62.4	4.2	12.2
	J774		
No particles	69.8	3.2	20.0
MgP	45.4	8.3	1.7
MgP02	24.5	14.8	7.6

DISCUSSION & CONCLUSIONS: Mg-F-C temporally decrease corrosion rate of Mg [2]. Reduction of corrosion rate implies the decrease in the release of ions and may increase biocompatibility. According with this, in a previous report with J774 cells, higher effect on LDH assays was detected in case of MgP than on MgP02 for ≥1 mg/ml. Nevertheless, present results showed that Mg-F-C affect viability of both cell lines assayed (MC3T3-E1 and J774). Macrophages seem to be more sensitive to MgP and MgP02 (1mg/ml) than MC3T3-E1. The presence of particles and the Mg-F-C on MgP02 also affect the cell cycle phases of the J774 severally.

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The electrophysiological effects of magnesium-based biomaterials on nerve cells

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INTRODUCTION: Magnesium-based biodegradable materials have shown great potential in clinic applications, even nerve conduits have been proposed recently. It is necessary to study the potential harm of Mg-based materials and their degradable products on nerve cells before wide applications. In neurophysiology, Mg^{2+} is a well-known N-Methyl-D-aspartate (NMDA) receptor blocking agent which may inhibit neuronal signal transmission. Here, the electrophysiological effects of Mg^{2+} and Mg-based material extracts were tested on microelectrode array (MEA) electrophysiology testing platform that are based on in vitro chick forebrain neuronal networks.

METHODS: Pure Mg and WE43 alloy extracts were prepared in culture medium according to ISO 10993-12 at 37 ℃ for 24 h. Dissociated forebrain neurons were harvested from day 9 chick embryos and plated into a PEI-pre-coated MEA (Multi-Channel System, Germany) with a density of approximately 2000cells/mm^2. Neurons and glia were co-cultured in neurobasal medium supplemented with B27 and GluMax for at least three weeks until the neuronal networks were spontaneously firing with synchronized, periodical, and relatively stable pattern, see Fig.1. During drug administration, the network activities were recorded for 30 min every day. The mean activities (spike rate) of three days before adding extracts, three days during administration, and three days after wash were set as “reference”, “exposure”, and “recovery” respectively. Medium added with 3 mM Mg^{2+} were used for comparison.

RESULTS: Both the *pH* values and [Mg^{2+}] of WE43 extract were higher than those of pure Mg, as shown in *Table.1*. 25% WE43 extracts, 25% Mg extracts, and 3 mM Mg^{2+} all significantly inhibited network activities during exposure and did not recover to reference level, as shown in *Fig.2*. The spike rate of 3 mM Mg^{2+} group were higher than extracts groups during exposure but lower after washout. There were no significant differences between 25% WE43 and 25% Mg.

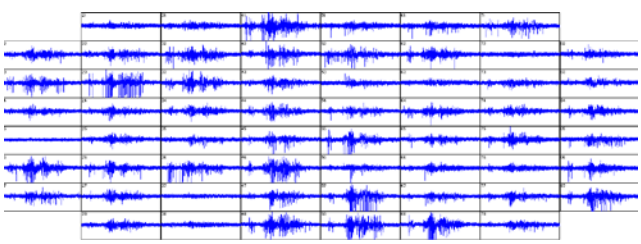
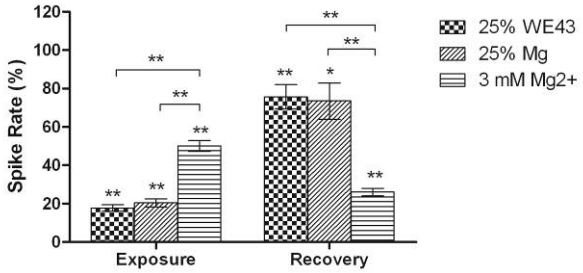


Fig. 1: A typical spontaneous neuronal network activity with synchronized firing pattern.

*Fig.2: The effects of 25% WE43 extract, 25% Mg extract, and 3 mM Mg^{2+} on spike rates of neuronal network electrophysiological activities(Mean ± SEM, *p < 0.01, **p< 0.001).*

Table. 1. The pH values and [Mg^{2+}] (mM) of medium, pure Mg extract, and WE43 extract.



	Pure Mg	WE43	Medium
pH	7.86±0.21	7.94±0.35	7.28±0.18
[Mg^{2+}]	10.60±0.40	12.08±0.24	0.88±0.06

DISCUSSION & CONCLUSIONS: The high concentration of Mg^{2+} extracted from Mg-based material had significant inhibition effects on neuronal electrophysiological activities. But the inhibition effects and recoverability of material extracts were different from those of the corresponding concentration of Mg^{2+}, which indicated that extracts may have more complicated mechanism than Mg^{2+} on neuronal network activities. Further differences need to be verified by molecular biology method.

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Cytotoxicity evaluation of ions released by a Mg-based alloy (ZEK100) in vitro: Comparison of the results obtained with different assays

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INTRODUCTION: Magnesium alloys are promising for temporary biodegradable implants in osteosynthesis, dental and cardiovascular applications. However, several reports showed that these bioabsorbable materials could induce adverse cellular effects *in vivo* due to the presence of metallic particles, pH increase during the biodegradation process, local hydrogen evolution, as well as excessive Mg ions. Recently we reported results obtained with Mg and ZEK100 discs immersed in CHO-K1 cell cultures [1]. We evidenced time- and space-dependent results showing the decrease of i) the viability and ii) the capacity of forming colonies of these cells. The aim of this work was to evaluate the contribution of metal ions released by this Mg-based alloy on the damage induced by the biomaterial. Both, extracts from metal discs of Mg and ZEK100, and binary and ternary combinations of salts of the alloying elements (La, Zn and Mg) were used in present studies. Comparison with previous results was made.

MATERIALS AND METHODS: Extracts (Exs) were obtained after the immersion of pure Mg (99.7%) and ZEK100 Mg-based alloy (0.96 wt.% zinc, 0.21 wt.% zirconium, 0.3 wt.% RE) disks into cell culture medium (CCM) for 24 h. An aliquot of the CCM without metal disk was incubated under the same conditions and used as control. Additionally, several sets of experiments were designed to assess the combined effect of metal ions. With this purpose solutions of the following salts and their combinations were employed: $MgSO_4$ (2.5×10^3 µM, 3.3×10^3 µM, 4.1×10^3 µM and 8.2×10^3 µM), 200 µM of $LaCl_3.7H_20$ and $ZnCl_2$ (50 and 100 µM) dissolved in the CCM. Cytotoxicity induced by metal ions was evaluated by Neutral red (NR) assays. Two set of experiments were performed. In one of them the cells were treated for 24 h with Exs and 24 h combined treatments employing solutions of metallic salts in the other set.

RESULTS: The SEM and EDS analysis of a Mg disk that had been immersed in the CCM showed a heterogeneous surface with areas in which EDS showed a Mg peak with only minor contribution of other elements while other regions depicted an important O signal with a relationship between the atomic percentages of O and Mg compatible with the presence of $Mg(OH)_2$. Unlike pure Mg, ZEK100 alloy spectrum also revealed contributions of Zn, P and Ca. These last two elements may be related to the presence of calcium phosphate that precipitated under the less aggressive corrosion condition. NR assay made with Exs containing metal ions released from Mg disks showed a decrease in lisosomal activity (80% of the control value) ($p < 0.001$). Conversely, no adverse effect was observed in case of ZEK100-Ex. In addition, different binary and ternary combinations of salts revealed no cytotoxic effect. These data contrast with the cytotoxic effect informed in a previous report related to metal disks that were immersed within the cell culture (Grillo et al. 2013).

DISCUSSION & CONCLUSIONS: Results demonstrated that the use of Exs and metallic salts are far from those obtained with the disks within the cell culture in which ion concentration is dependent on the contact period and on the distance from the source of ions (closer to the clinical situation). They also highlight the importance of studying the biomaterial-cells interface where different local changes (pH, hydrogen burbles) occur and strongly affect biocompatibility. Consequently, it is very important to choose the appropriate methodology to evaluate *in vitro* cytotoxicity as a previous step to conducting *in vivo* assays.

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Novel magnesium-based stent biomaterials with anti-corrosion and drug-eluting coatings

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INTRODUCTION: Magnesium-based drug-eluting stents present the latest generation of cardiovascular stents. However, the low corrosion resistance of the scaffold and unideal drug release profile are the main drawbacks of such novel stents. To overcome these problems, a three-layer coating system was introduced to magnesium-based alloys. A dynamic corrosion and drug release test platform was used to detect the degradation and drug release profile. This dynamic test system provided a better mimic of in vivo scenarios compared to static systems. In addition, we also examined the biocompatibility of the coatings by cell toxicity tests of endothelial and smooth muscle cell cultures.

METHODS: The magnesium AZ31 samples were coated with a three-layer coating, i.e., dextran-polyglumatic acid-dextran. Sirolimus was loaded within the first and the third layers of dextran. The thickness of polyglumatic acid varied depending on the solution concentration (0%, 3%, 5%, and 9% mg/ml, respectively). The dynamic corrosion and drug release kinetics test platform consisted of pump, tubing and liquid container, and Hank's buffer was used as the circulating solution. High-performance liquid chromatography (HPLC) combined with ultraviolet detection (UV) was used to measure the concentrations of sirolimus. Endothelial cell and smooth muscle cell culture, and platelet adhesion tests were performed to explore the biocompatibility of the coatings.

RESULTS: Compared to uncoated AZ31, the corrosion resistance of coated samples was significantly enhanced. Moreover, coated samples had better biocompatibility. The drug release time was correlated with the thickness of polyglumatic acid layer. A longer release time was observed for samples with thicker polyglumatic acid layer. The released sirolimus could effectively inhibit the proliferation of smooth muscle cells.

DISCUSSION & CONCLUSIONS: The dextran-polyglumatic acid-dextran coating effectively increased the corrosion resistance, and had better biocompatibility compared to uncoated control. The sirolimus release time can be controlled by altering the thickness of polyglumatic acid layer.

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Effect of magnesium extracts on the chondrogenic differentiation of HUCPV cells

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INTRODUCTION: Magnesium (Mg) and Mg alloys are promising candidates to be employed as resorbable implants in orthopaedics [1]. Previous studies have showed that Mg based materials are suitable for the treatment of cartilage disorders [2], where combination with cell therapy is necessary. Furthermore, for the treatment of fracture in growing bones in children, it is important to consider the influence of the degradation on the growth plate (mainly constituted by cartilaginous tissue). For both purposes, human umbilical cord perivascular cells (HUCPV) are a new source of mesenchymal cells that exhibit a high potential to be employed for this purpose, due among other reasons, to their strong capability to differentiate into osteoblast and chondrocyte [3].

METHODS: The influence of different concentrations of Mg in the extracts over HUCPV cells proliferation was determined by quantifiying the cells at different time points. Chondrogenic differentiation of HUCPV was induced in a 3D model with alginate (*figure 1*) and subsequent micro-pellet formation during several time periods. The effect of Mg extracts on the differentiation process was evaluated analysing the expression of chondrogenic gene markers as type II collagen (COL2A1), aggrecan (ACAN) and [SRY (sex determining region Y)-box 9], (SOX9) were evaluated by real time polymerase chain reaction (rtPCR), both in short-term (from 1 to 21 days)and long-term (up to 42 days) evaluation. Regarding the effect over the synthesis of extracellular matrix (ECM) Type II (pro) collagen (COL2) release to the supernatant was tested after 11 (control) and 42 days of cell differentiation induction and glucosaminoglucans (GAG) content in the ECM of the micro-pellets was determined by 2-9-dimethyl-methylene blue (DMMB) colorimetric assay, and the radio GAG/DNA content (determined by fluorescence assay with bisBenzimide) was calculated.

RESULTS: HUCPV showed higher proliferation with addition of Mg in a concentration around 3 mM. No effects decreasing proliferation were detected up to a concentration 6 mM. Expression of chondrogenic gene markers of differentiated cells from pellets cultured with Mg extracts was higher than the controls (cells cultured without Mg).

Release of COL2 into supernatants, as well as the ratio GAG/DNA were higher when cells were cultured under the influence of Mg extracts during 21 days.

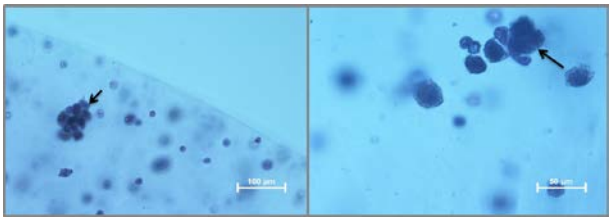

Fig. 1: Chondrogenic differentiation of HUCPV cells in a 3D alginate model. Staining with dimethyl-methylene blue (DMMB) of sulphated GAG present in the ECM.

DISCUSSION & CONCLUSIONS: Those results indicate that addition of (even) diluted Mg extract induces HUCPV proliferation and stimulates cell differentiation into chondrocytes and synthesis of cartilage-like ECM.

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Effect of magnesium particle shape on *in-vitro* degradation kinetics of novel PLA/Magnesium composites

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INTRODUCTION: In some cases, metallic prosthetic devices must be removed by a second surgical procedure. Biodegradable devices appear as an alternative to avoid this risky and costly practice. One of the main drawbacks of bioresorbable polymers is their low mechanical strength that leads to the need to oversize the implants. With magnesium base materials, on the other hand, hydrogen may form so quickly that became accumulated around the implant at a rate difficult to deal with by the tissue [1, 2]. In the present work, an alternative material, which consists in the incorporation of Mg particles into a polymer matrix [3], has been processed and the degradation kinetics studied as a function of the shape of the Mg reinforcing particles.

METHODS: 10% weight of i) commercial purity irregular shaped and ii) argon atomized spherical Mg particles of < 50µm were introduced in poly-D-L-lactic acid (PLDA) matrices by extrusion and compression moulding of the composite filaments into cylinders of 9 mm high and 6 mm dia. Degradation was monitored in triplicate during up to 28 days. Hydrogen release in a PBS solution was measured twice a day, and pH variation in distilled water and in PBS was recorded each hour for the first 10 h and then once a day. The solutions were fully renovated once a week.

RESULTS: Figure 1 shows the cylinders after 7 days of immersion in PBS. It is remarkable the different aspects of the two Mg reinforced composites. The irregular shaped particles lead to the formation of deep and long cracks and the enlargement of the specimens, whereas the spherical particles neither promote crack formation nor modify the cylinders shape, which present a similar aspect after 28 days of immersion (not shown). This difference is accompanied by a much higher hydrogen release of the composite with irregular Mg particles, which amounts to 2.44 ml H_2/cm^2 after 7 days of immersion (0.36 ml H_2/cm^2/day), more than six times the amount of 0.37 ml H_2/cm^2 released by the spherical reinforced composite (0.05 ml H_2/cm^2/day). This better behaviour of the latter is also appreciated after 28 days of immersion: 6.76 versus 5.02 ml H_2/cm^2 (0.24 versus 0.18 H_2/cm^2/day).

Fig. 1: Photographs of the three specimens of each composite after immersion during 7 days in PBS solution.

In PBS, pH stays stable at a value between 7.2 and 7.4 for both composites. In water, pH rises sharply during the first 1.5 days up to about 9.4. This increase is slightly slower for the composite with spherical Mg particles.

DISCUSSION & CONCLUSIONS: The shape of the Mg particles that act as reinforcement of biodegradable implants plays a crucial role in their degradation rate. Spherical particles promote much better behaviour than irregular ones, probably due to their much smaller surface area to volume ratio.

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ACKNOWLEDGEMENTS: MAT2012-37736-C01 **(**MICCIN) for financial support, CSIC and European Social Fund for JAE-I3P Grant (SC Cifuentes). Special thanks to Prof. V. Lorenzo (UPM) for his help with the processing.

Influence of SaOS-2 cells on corrosion behaviour of cast ZM21 magnesium alloy

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INTRODUCTION: Mg alloys are promising materials for biodegradable orthopedic applications. However, control of Mg alloys biodegradation is crucial for their success as implant devices. In this study, corrosion behavior of ZM21 was evaluated in the cell culture condition by electrochemical impedance spectroscopy (EIS) technique. Influence of cells cultured on ZM21 on its corrosion properties was also investigated.

METHODS: Cast ZM21 alloy (2.0 wt% Zn, 0.98 wt% Mn) samples (10x5x1.5mm) were polished with SiC grinding paper (14µm) damped with 99.9% ethanol, ultrasonically cleaned with acetone, and sterilized with ethylene oxide gas (EOG). Degradation behavior of samples was analyzed by EIS. The tests were conducted using a potentiostat equipped with frequency response analyser with a typical three electrode system. The sample with 0.264 cm^2 of exposed area was used as the working electrode, with a Ag/AgCl(3MNaCl) reference electrode and a platinum counter electrode. The electrolyte was a Dulbecco's Modified Eagle Minimum Essential Medium supplemented with 10% (v/v) fetal bovine serum (DMEM+10%FBS) maintained at 37^{0}C, 5% CO_2 and pH 7.5. In some experiments, human osteosarcoma cell line (SaOS-2) was inoculated at a density of 40 000 cells/ml in 5 ml of a DMEM+10%FBS. EIS was performed over the frequency range of 10^5–10^{-2} Hz at 5 mV AC amplitude under cell culture condition (37°C, 5% CO_2) up to 48h.

RESULTS: The Nyquist plots for the ZM21 alloy without/with cells after 2, 6, 24 and 48h culture are shown in Fig.1a and 1b, respectively. For samples without cells, capacitive loop expanded along the incubation time. However, in case of the sample with cells reduction of capacitive loop was noticed after 6h of culture. During the culture period, the pH of the medium slightly decreased for samples with cells (from pH 7.9 after 2h to 7.75 after 48h), in comparison to those without cells, where pH was around pH 7.9.

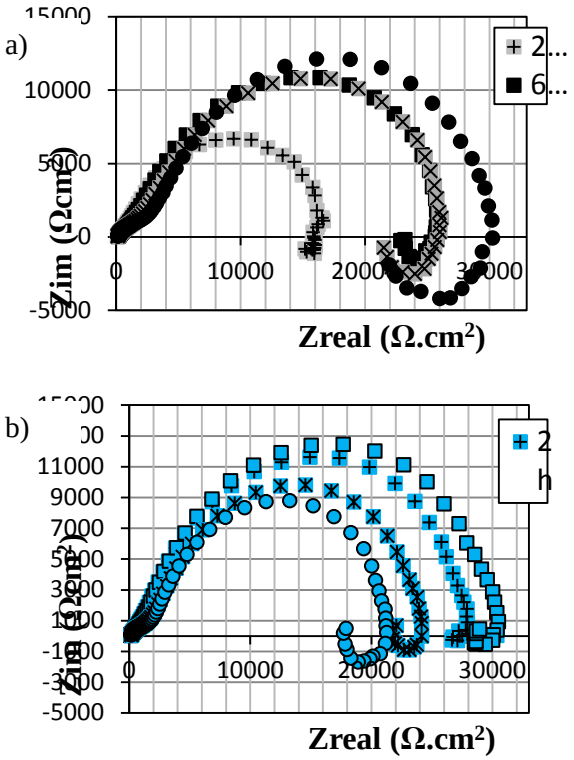

Fig. 1: Nyquist diagram of samples a) ZM21 alloy in D-MEM +10% FBS, b) ZM21 alloy in D-MEM + 10% FBS supplemented with 40 000 cells/ml.

DISCUSSION & CONCLUSIONS: The expansion of the capacitive loop of ZM21 without cells along the culture period suggests the decrease in the corrosion rate of ZM21 in the medium, due to the formation of insoluble salt layer in the medium, which acts as a barrier for the diffusion of water molecules or other ions. After several hours of culture, the presence of cells reduces the formation of insoluble salt barrier due to the lower pH of the medium as a result of cell metabolic reaction [1], which accelerates corrosion of ZM21 as shown as the reduction of capacitive loops in Fig. 1b.

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Proteomic approaches for studying bone tissue sections

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INTRODUCTION: One of the valuable techniques for fast and direct spatial analysis of biomolecules in biological tissues is MALDI mass spectrometry imaging (MSI) [1]. MALDI-MSI is especially remarkable for researching for disease markers by analyzing tissue sections in healthy and diseased conditions [2]. Here, we analyzed the spatial protein composition of formalin-fixed paraffin embedded (FFPE) and formalin-fixed plastic embedded FFPlE) bone tissues. Formalin fixation chemically causes modifications in proteins thus makes the identification of proteins from these types of tissues difficult [3]. In addition in this study, we established a method for proteome analysis of FFPE bone tissue sections.

METHODS: At the first step, tissue sections were mounted onto indium-tin-oxide (ITO)-coated conductive glass slides, deparaffinized or deplastified. Then, a trypsin solution was sprayed on the tissue slides with a MALDI-matrix spraying device (Image-Prep: Bruker Daltonik) followed by incubation at 37 ºC for three hours. The matrix solution DHB was sprayed on the tissue sections with the MALDI-matrix spraying device and was dried. Finally, samples were measured by MALDI-MSI (autoflex speed, Bruker Daltonik). Data analysis was done with flexcontrol 3.3, flexanalysis 3.3, and fleximaging 2.1 Imaging (Bruker Daltonik).For the next step, 20 consecutive tissue sections from bone tissue were sliced in 6µm thickness, and transferred to glass slides, deparaffinized and deplastified. Subsequently, tissue areas of interest were separated and transferred from the slides to defined reaction vials, and finally incubated with trypsin. Then, the tryptic peptides were analyzed by LC-Q-TOF, and LC-TOF-TOF. Protein identification was done with mass spectrometric data processing by OpenMS and comparing the data with a protein database (SwissProt) by a search engine (MASCOT).

RESULTS: We studied the spatial distribution of bone tissue slices of mice and rat. In the MALDI-MSI spectra many signals representing tryptic peptide ions are present. Also, we established a protocol for identification of proteins with which we are able to identify approximately 1000 proteins from 20 sections of FFPE tissue.

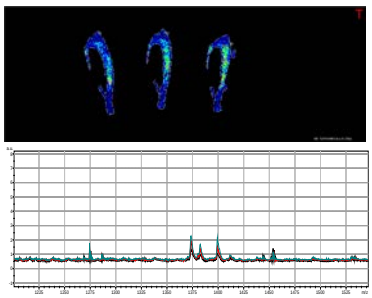

Fig. 1: *The Image shows the intensity of a selected signal in the MSI spectra, m/z:1274*

DISCUSSION & CONCLUSIONS: The spatial distributions of the signals in the results of measurement by MALDI-MSI can be clearly allocated to morphologically distinct areas. Therefore MALDI-MSI gives valuable information about the distribution of different proteins. Moreover, the proteins identified by proteome analysis of tissue sections served as a database for assigning the identity of peptides observed in previous MSI studies of FFPE tissue micro arrays.

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Evaluation of cell damage produced by Fe ions released as degradation products of biomaterials. Influence of pH changes.

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INTRODUCTION: Pure Fe and some of its alloys have been proposed for the manufacture of temporary stents because they are susceptible to a fast corrosion in biological media [1]. However, the release of Fe ions during dissolution may cause side effects like oxidative stress and inflammation in the tissues in contact with the implant (Fig. 1). Despite its importance, the mechanisms by which these deleterious effects are produced have not been sufficiently described in the literature. In this work, the effect of Fe^{2+} and Fe^{3+} ions and pH changes on cultures of CHO-K1 cells was evaluated.

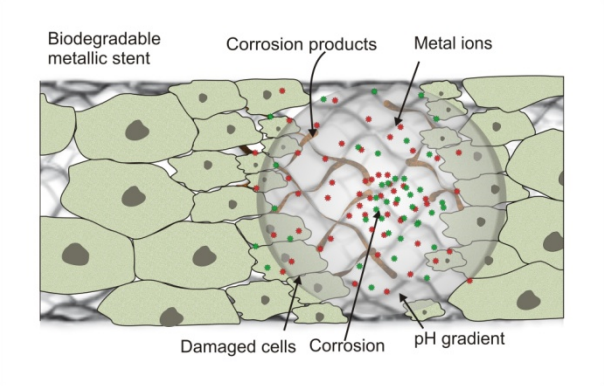

Fig. 1. Diagram of changes during Fe stent dissolution in contact with biological medium and cells.

METHODS: CHO-K1 cells were exposed to various concentrations of Fe^{2+} and Fe^{3+} salts (1-5 mM range) and cellular mitochondrial activity (MTT technique), oxidative damage to lipids (TBARS determination) and intracellular Fe (bathophenanthroline technique) were determined.

To evaluate "Fe effect" the Fe^{+2} solutions were prepared in acid culture medium (CM, pH <7) to increase the solubility of the salt, then it was diluted with alkaline CM (pH ≈ 9) until the desired concentration and pH were reached. Fe^{+3} solutions were prepared with CM at its usual pH (pH ≈ 8.4). After the addition of Fe salts different pH values were obtained. The "pH effect" was determined using CM adjusted to pH values similar to those reached after the addition of Fe salts.

RESULTS: The cellular mitochondrial activity related to pH was compared with that owed to Fe. Results showed that in all cases ("pH", "Fe^{2+}" and "Fe^{3+}" effects) the mitochondrial activity was lower than in control cells. In 4-5 mM Fe^{2+} the detrimental effect on mitochondrial function was higher than in the solution of similar pH. Consequently, the decrease of mitochondrial activity for 1-3 mM concentration range may be exclusively attributed to the decrease in pH. The effect of "Fe^{3+}" was greater than the effect caused by the pH alone for 3-4 mM concentrations range. Interestingly, higher levels of intracellular Fe at this concentration range compared to control cells were obtained.

Significantly higher TBARS values were obtained in the case of "Fe^{2+}" and "Fe^{3+}" than in the absence of Fe ions, indicating that in the presence of the metal ions higher oxidative damage to lipids is found, independently of the pH value (in the 5.6-8.4 pH range).

DISCUSSION & CONCLUSIONS: Results indicate that during Fe stent dissolution cytotoxicity effects may be found. They depend on diverse variables such as pH changes, formation of precipitates and Fe ions concentration. Conversely, oxidative damage to lipids is only observed in the presence of Fe. These changes occurring at the biomaterial/biological medium interface during degradation of Fe may affect the viability of cells in the vicinity of the implant.

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ACKNOWLEDGEMENTS: Authors acknowledge the financial support from UNLP, CONICET and ANPCyT.

The application of biodegradable magnesium screw in hip surgery

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INTRODUCTION: Prospective studies have been done for hip surgery with biodegradable pure magnesium screws. Meanwhile, follow-up evaluation on the stability, degradability and safety of biodegradable magnesium screw has been reported for the first time.

METHODS: Forty patients with internal fixation of biodegradable pure magnesium screws have been chosen from february 2013 to April 2014. We used magnesium screws in three surgical techniques: vascularized bone transfer for osteonecrosis of the femoral head (ONFH), titanium cannulated screws internal fixation combined with vascularized bone graft for femoral neck fracture and acetabular defect repairs for hip replacement. Vascularize bone graft taken from iliac or greater trochanter area was fixed to the femoral head necrotic area in ONFH cases or femoral neck fracture site in fracture cases by magnesium screws. In hip replacement cases with severe acetabulum defect while the remain acetabulum would not provide enough bone coverage for the acetabular prosthesis, magnesium screws were used for fixation of the bone graft for reconstruction of the acetabulum. However, none of the magnesium screws were applied for weight bearing zone of the hip.

RESULTS: In this study, patients were followed up for 6-12 months (average 8 months).At last follow-up, all screws have not been degraded completely. Postoperative liver and kidney function, calcium and phosphate ions are in the normal ranges, while one patient's serum magnesium ions increased to 1.1mmol / L (normal range 0.73-1.06 mmol/L) after 14 days of the operation, and it became normal without treatment after 18 days of operation. The longest follow-up time was 12 months, there were no gas production, allergic reaction, osteolysis and other adverse reactions, no secondary fracture, prosthesis loosening occurred in the degradation process, bone fixation are satisfactory without bone graft displacement.

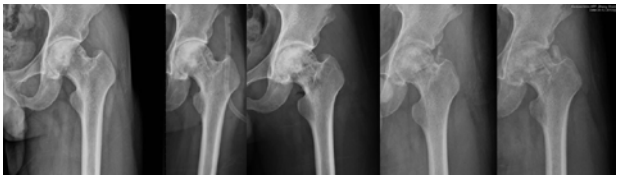

Fig. 1: Pre and postoperative Imaging (posterior-anterior) of ONFH.

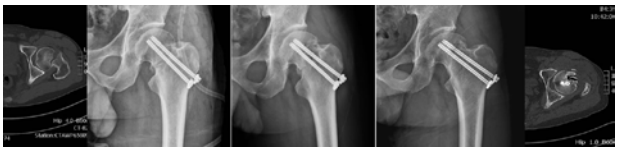

Fig. 2: Pre and postoperative Imaging (posterior-anterior) o f femoral neck fracture

DISCUSSION & CONCLUSIONS: The radiographic and clinical evaluation of cases of this study demonstrated that biodegradable pure magnesium screws had no systemic toxicity, osteolysis and inflammatory reaction. Biodegradable pure magnesium screws in the non-weight bearing area could satisfied local biological strength requirements. The postoperative hip function of using degradable pure magnesium screws are comparable to traditional techniques, but one of the most advantages of these new screws is that it requires no additional operations. However, the follow up of this study was relatively short, and we do not know how long it will take for the screws to be biodegraded completely, future studies should be focused on long-term consequences.

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Long-term in vivo degradation behaviour of Mg alloys WZ21 and ZX50 – A micro CT study

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INTRODUCTION: Stainless steel, titanium and cobalt based alloys offer very suitable biomechanical properties for fracture stabilization. In comparison to these commonly used materials, biodegradable magnesium will save a second operation for implant removal and promises to support the fracture healing process by the avoidance of stress shielding due to its elastic modulus similar to human bone.

Furthermore, challenging facts when considering magnesium as material for orthopaedic load bearing devices, is the production of hydrogen gas during degradation and the degradation time itself. Latter is also responsible for the maintenance of stability within the fractured bone. These two factors exhibit a strong correlation and need to show a balance between optimal stability during bone healing process and moderate gas production. Restitutio ad integrum of bone structure after implant's full degradation should be achieved after 12-15 months[1]. Our group has already investigated the degradation behaviour of two Mg alloys (fast degrading ZX50 and slow degrading WZ21) over a period of 9 months[1]. This follow-up study aims to evaluate the long-term degradation behaviour of these alloys and the response of the injured bone between month 9 and 24.

METHODS: Two different Mg alloys were investigated: ZX50 (Mg-5Zn-0.25Ca-0.15Mn-0Y); WZ21 (Mg-1Zn-0.25Ca-0.15Mn-2Y). For this study cylindrical pins with a diameter of 1.6 mm and a length of 8 mm were used. Eight pins per alloy were implanted through a transcortical drill in both femurs of male Sprague-Dawley® rats. Continuous micro CT monitoring of degradation behaviour and histological examinations were performed at time points 9, 12, 15, 18 and 24 months. Volumes and surfaces were quantified using Siemens Inveon Acquisition Workplace Ver. 1.2.2.2.

RESULTS: The degradation behaviour respectively to implants volume and surface was measured at each timepoint. ZX50 did not show any degradation residuals after 9 months and a completely recovered and remodelled bone was noticed. This corresponds to the results of Kraus et al [1].

So this study focuses on the ongoing degradation of WZ21. Changes in pin volume, surface and gas emission behaviour were examined in vivo. Complete degradation of WZ21 implants and restitutio ad integrum of the bone structure appears after 24 months of study period. WZ21 implants showed a production of hydrogen gas bubbles until month 12 without any visible interference on bone regeneration. Degradation rate seemed to be slightly delayed in the area of cortical bone compared to the medullar cavity. After 18 months, some implant residuals were noticed, after 24 months the bone was fully remodelled without any corrosion products or defects. This was observed via micro CT and also during histological examination. None of the implants showed pathological increase of inflammatory cells.

DISCUSSION & CONCLUSIONS: Micro CT and histological analysis of ZX50 did not show any noticeable long term defects in the treated bone during this study period from month 9 to month 24. The slight degradation rate and moderate gas production during long-term evaluation, suggest WZ21 to be a very suitable material for orthopaedic application. Also complete remodelling and restitutio ad integrum of the injured bone after month 24 aspects in its favour. Nevertheless it is unclear how the included REE Yttrium actually influences physiological reactions in the skeleton.

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In vivo degradation and biocompatibility of linear cutter staples made of high purity magnesium

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INTRODUCTION: Magnesium can be used as biodegradable surgical devices due to good biocompatibilities [1]. However, it has not been systematically investigated in this field, e.g. linear cuter staples. In this study, high purity magnesium staples were fabricated and then were used to cut and close pig's stomach aiming to study the in vivo degradation and biocompatibility.

METHODS: High purity magnesium staples with diameter of about 0.3 mm were fabricated (Fig.1a), which can be totally degraded in vitro becoming homogeneous hydrates (Fig.1b). Then the staples were used to cut and close pigs' stomach (Fig.1c). The pigs were sacrificed after 9 weeks to investigate the revival of the stomach and the biocompatibility according to HE methods.

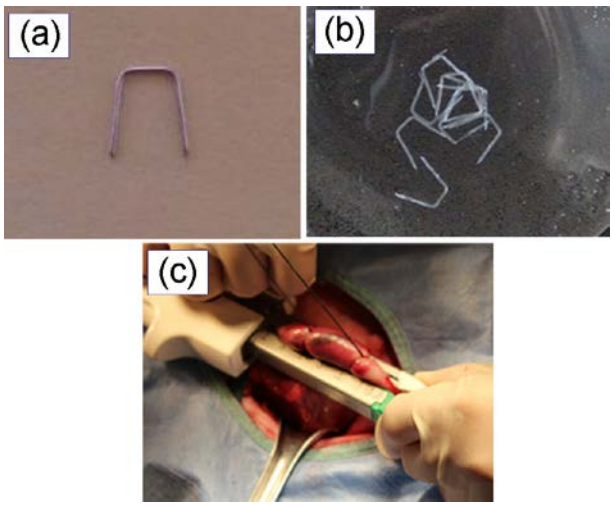

Fig. 1: Mg staple (a) with homogeneous degradation behaviour (b) and surgical process on the pigs' stomach (c).

RESULTS: The cutting parts can be totally closed without any leaking. The wound was healed after 9 weeks (Fig.2a) exhibiting low degradation rate with about 95% remains (Fig.2b). No cracks can be found, and no inflammation or other side-effect can be seen (Fig.2c), suggesting good biocompatibilities.

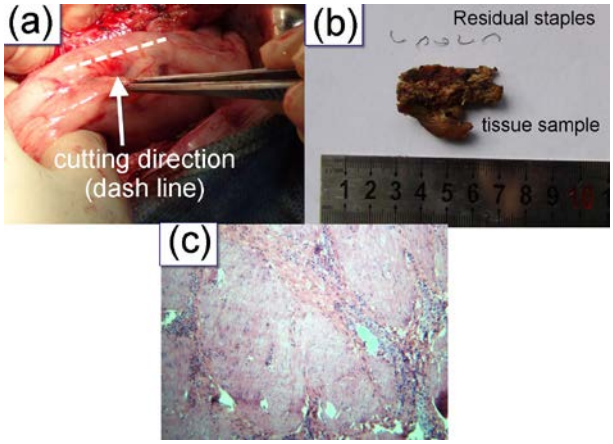

Fig. 2: Revival of cutting part (a), residual staples (b) and HE stained tissues (c).

DISCUSSION & CONCLUSIONS: The linear cutter stapler is a very important surgical device in the general surgery field. However the present staples are made of titanium and Ti alloys, which can not be absorbed by the human being causing negative effects. Therefore, it is quite attractive to develop absorbable staples with satisfied treatment results. Because the diameter is very thin it is vital to garantee homogeneous degradation behaviour to avoid sudden failure. And it is also a key point to maintain the sealing strength of the staples otherwise leaking will lead to fatal acute epiploitis. In the present study a kind of staples with homogeneous degrading rate was developed and in vivo pig experiments showed that the cutting part can be sealed effectively without any negative effects (for example, inflammation). Therefore it is a promising biodegradable linear cutter staples for general surgeries.

REFERENCES: [1]AC. Hazi et.al (2011) *Materials Science and Engineering C* **31**:1098–1103.

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Preclinical and clinical study of Mg-Ca-Zn alloy

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INTRODUCTION: Preclinical and clinical study of small bone screw made of Mg-Ca-Zn alloy was performed to evaluate the safety and efficacy for human use in hand fracture. Preclinical study showed excellent biocompatibility of this bone screw. After confirming toxicological safety of Mg-Ca-Zn alloy in the preclinical study, the protocol of clinical study for human hand fracture was developed. Initial two cases of human clinical study, which have finished 1 year follow-up recently, showed perfect fracture healing and restoration of hand function without any complication.

METHODS: After casting and extrusion of Mg-Ca-Zn alloy, the small bone screw of the alloy was fabricated through machining process in CNC lathe. Preclinical study including, cytotoxicity, sensitization, acute systemic toxicity, genotoxicity, and irritation tests were performed according to ISO 10993 series standard. Then clinical protocol for human clinical study was developed to evaluate the effectiveness and safety of the biodegradable screw made of Mg-Ca-Zn alloy. Primary endpoint was bone union in fracture site at 6 months after surgery. PROM (Passive Range of Motion), TAM (Total Active Motion), Power, DASH (Disability of the Arm, Shoulder and Hand) scale and pain were also included in the protocol as secondary endpoint. Following this protocol, the small bone screws made of Mg-Ca-Zn alloy were implanted to the patient with hand fracture. Initial two cases with 1 year follow-up were evaluated with radiography and hand function.

RESULTS: All of the preclinical study related with biocompatibility showed minor or no toxicity of Mg-Ca-Zn alloy. The initial two cases which finished 1 year follow-up in clinical study showed mild degradation of small bone screw, perfect bone union in fracture site, and restoration of hand function in 6 months after surgery. After 1 year follow-up, more degradation of bone screw was observed without any complication in all cases.

DISCUSSION & CONCLUSIONS:

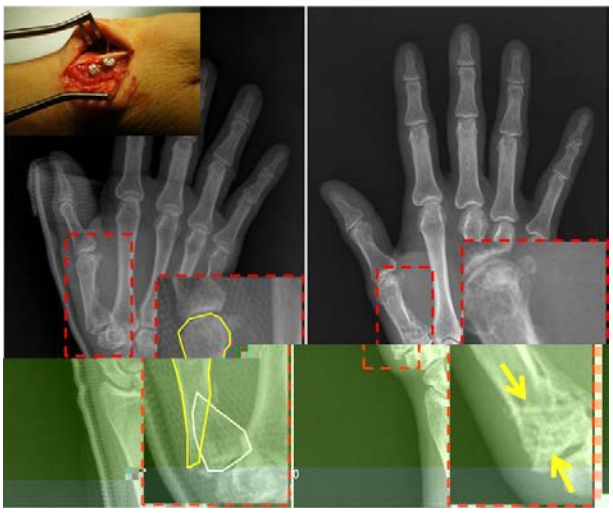

Fig. 1: Images of hand at fracture (left), 6 months after surgery (right)

In previous study[1,2], implantation test of Mg-Ca-Zn alloy, with 1 year follow-up, showed good biocompatibility in all time points and higher strength comparing with biodegradable polymer was observed. In this study, the small bone screw of Mg-Ca-Zn alloy showed minor or even none toxicity in cytotoxicity, sensitization, acute systemic toxicity, genotoxicity, and irritation tests. Therefore, this alloy is considered biocompatible enough to use *in vivo*. Although mechanical strength of Mg-Ca-Zn alloy is lower than titanium, stainless steel, and chrome cobalt alloy, the result of initial two cases of human clinical study showed safety and effectiveness for use of human bone fracture.

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The Korea Healthcare Technology R&D Project, Ministry of Health and Welfare, Republic of Korea; contract grant, number: A101942

Degradable magnesium plates and screws for bone fracture fixation

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INTRODUCTION: Each year there are over 6 million bone fractures in the U.S., often requiring internal fixation [1]. Traditionally, internal fixation devices have been made with permanent metals like titanium. Resorbable polymer devices have also been developed; however mechanical limitations often bar them as viable options for load-bearing applications. Uniquely, magnesium (Mg) alloys provide a balance of degradation and strength, making them ideal candidates for bone fixation devices.

METHODS: Mg fixation plates and screws were designed and tested using a New Zealand white rabbit ulna fracture model. Fixation plates and screws were machined from 99.9% pure Mg (Goodfellow, Coraopolis, PA) and sterilized with gamma radiation. Ulnar osteotomies (0.5-1mm thick) were created and secured with Mg plates and screws. Forearms were left un-casted and were harvested after eight and 16 weeks.

MicroCT was used to evaluate device degradation and bone formation. Devices were scanned before implantation and after eight and 16 weeks, and assessed as previously described [2]. In addition, Toluidine Blue histological staining was performed to visualize bone morphology.

Three point bend tests were used to evaluate the relative ulnae strength after 16 weeks [3]. Test parameters were modified from previous studies [3], employing a loading speed of 5mm/min and stop point of 0.5mm flexural extension. Flexural load at maximum extension was recorded for each sample and compared to healthy controls.

RESULTS: After eight weeks, the Mg screw volume was reduced by 4.41±0.49 mm^3. At the same time, 3.35±0.60 mm^3 of corrosion product was produced at the surface. Based on this Mg volume change, the *in vivo* corrosion rate was calculated to be 0.40±0.04 mm/year.

Despite the presence of ongoing degradation, fracture healing was observed to be uninhibited. MicroCT and histological staining showed cortical bone union at eight weeks, with additional remodelling by 16 weeks. In addition, abundant new bone formation was observed around the Mg devices. A significant increase in overlying bone formation was observed from eight to 16 weeks ($p=0.001$), with 100.20±33.80 mm^3 of new bone that was formed over this period.

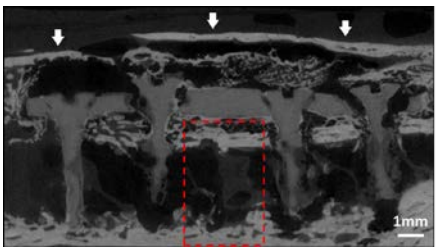

Figure 1: MicroCT slice of ulna with Mg fixation device after 16 weeks. Red box outlines fracture site, and white arrows highlight new bone formation around the Mg device.

Bend test results revealed a slight, though not significant, increase in flexural load for healed ulnae fixed with Mg devices compared to intact controls.

DISCUSSION & CONCLUSIONS: These results demonstrate the potential for Mg fixation devices in a semi-loaded fracture environment. Specifically, we observed uninhibited fracture healing with abundant local bone formation when using degradable Mg devices.

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Magnesium/PLGA Composite Scaffolds for Improved Bone Regeneration

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INTRODUCTION: 300,000 dental bone grafting procedures are performed annually in the US [1]. Synthetic bone grafting material is widely used due to the drawbacks of autografts and allografts; however, currently available synthetic bone grafting materials still have limitations [2]. Our goal was to develop a porous metallic magnesium particle / poly(lactic-co-glycolic acid) composite that would couple the osteoconductive properties of Mg with the versatility of PLGA. This study evaluated Mg/PLGA scaffold microstructure, degradation, cell attachment and biocompatibility in a canine socket preservation model.

METHODS: Cylindrical scaffolds were fabricated using a solvent casting, salt leaching method [3]. Molds were filled with a combination of magnesium powder, and/or sodium chloride particles. PLGA was dissolved in dichloromethane at 10% or 40% w/v and then added to the molds. Salt washout was performed in ddH2O, followed by lyophilisation and gamma sterilization. Scanning electron microscopy was performed to assess the microstructural properties of the scaffolds before and after BMSC seeding. *in vitro* degradation assessments were performed by measuring media pH and magnesium release using ICP-AES. Finally, a pilot *in vivo* biocompatibility assessment was performed in a canine socket preservation model.

RESULTS: Scaffolds were successfully synthesized with varying porosities. SEM revealed a complex morphology of magnesium particles bonded together by PLGA. The 50% Mg and 25% Mg scaffolds appeared more porous than the 100% Mg scaffolds. Additionally, BMSCs were visible on the surface of the scaffolds 12h and 7d after cell seeding (Fig. 1). Magnesium-containing PLGA scaffolds did not exhibit the decrease in pH observed in PLGA Only (0% Mg) scaffolds. Magnesium release into the medium increased with increasing Mg amount in the scaffold. 100% Mg scaffolds were still releasing magnesium into the medium at 4 weeks, while 50% Mg scaffolds were releasing a much lower amount and 25% Mg scaffolds had finished releasing magnesium.

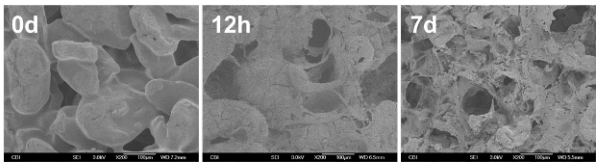

Fig. 1: Images of 100% Mg/PLGA scaffolds as-synthesized (0d), 12h and 7d after BMSC seeding.

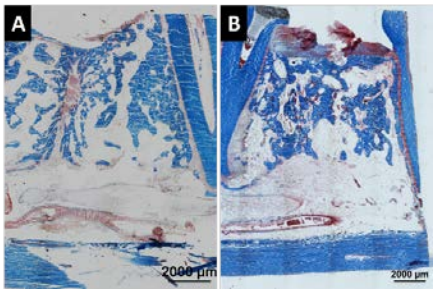

Fig. 2: H&E staining of explanted sockets containing Mg/PLGA at A) 8wk and B) 16wk

Implantation of scaffolds into the canine premolar socket resulted in increased bone formation and better preserved bone height as measured with microCT. Histological analysis at 8wk and 16wk showed bone formation and no signs of chronic inflammation around the defects.

DISCUSSION & CONCLUSIONS: Porous Mg/PLGA scaffolds were successfully synthesized and exhibited *in vitro* and *in vivo* biocompatibility. Increasing Mg composition in the scaffold increased Mg release time during degradation. Future steps will include assessment of early stage inflammatory responses to implantation of the Mg/PLGA scaffolds.

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Embolic risk mitigation in absorbable implants by composite design

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INTRODUCTION: The aim of this study was to investigate the *in vitro* degradation behaviour of differentially absorbable composite wires and to quantify embolic risk amongst candidate designs. Well-engineered absorbable medical device implants (e.g. stents) hold the promise of providing appropriate therapy and a consummate endpoint comprising only natural tissue that is mechanically and chemically functional. This beautiful reality stands within reach and yet there are significant risks that will be overcome prior to fruition. For example, in stenting of arteries or veins, particle fallout over the therapeutic course which may raise an embolic event, must be intentionally precluded by thoughtful and proven design. It is known that the localized corrosion behaviour is at least a function of materials selection and processing and also the final *in situ* materials stress state [1-2]. The design of this work is towards a composite structural approach to alter the working stress state, understand resultant *in vitro* corrosion, and begin to inform an embolic risk mitigation strategy with *in vivo* utility.

METHODS: Monolithic and drawn-filled tube (DFT) composite wires were produced with an external iron – 35 wt.% manganese (FeMn) alloy surface. Fig. 1 shows the schematic wire-transverse cross sections of the as-tested 200 µm filaments. Pure electron-beam grade tantalum (ASTM F560, RO5200 grade) and binary NiTi (Ni-49.2 at.% Ti) were used in the composite wires and manufactured using conventional medical wire processes as discussed elsewhere [3].

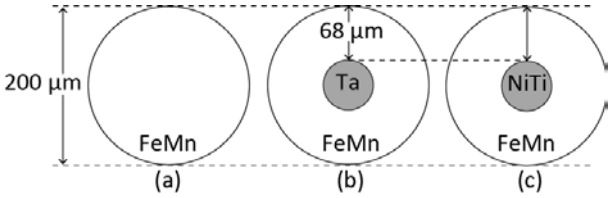

Fig. 1: As-tested 200 µm diameter wire sections from left (a) monolithic Fe-35 wt.% Mn (FeMn), (b) tantalum core FeMn composite, (c) elastic NiTi core FeMn composite.

All wires were coiled to an initial surface strain of approx. 10% by wrapping the 0.20 mm wires in a uni-filar coil arrangement around a 1.8 mm diameter stainless steel mandrel. Here, surface strain was calculated by neglecting out-of-plane curvature as: $\varepsilon_{surface} = d / (d + D)$. Corrosion testing in either phosphate-buffered saline (PBS) or bovine serum (BS) was conducted by stirred immersion of the free, no-load coil, or under an axial stress ranging from 100 to 500 MPa.

RESULTS: Fig. 2 provides a representation of the stress-corrosion-protective impact of surface compression resulting from a well-bonded and elastic NiTi core element. This protective behaviour is active until sufficient external stress overrode the internal composite stress balance.

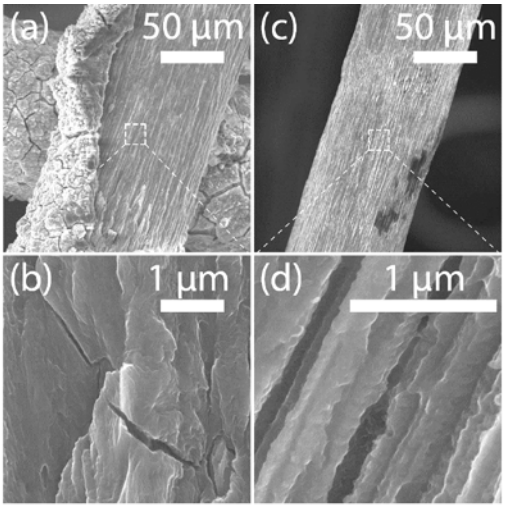

Fig. 2: Representative images of surface corrosion morphology of monolithic, coiled FeMn (a,b) and elastic core DFT (c,d) after 48 hours of no-load exposure to 37°C bovine serum.

DISCUSSION & CONCLUSIONS: This work has shown that a beneficial mechanical stress balance can be achieved through compositing to guard against the potentially embolic event of stress-corrosion cracking in thin FeMn wires for vascular devices. Similar possibilities exist for future composite Mg-alloys to be used in load-bearing orthopaedic applications. Conclusions given here were only partially drawn at the time of abstract composition. Additional data will be available at the August 2014 event.

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Peri-implant assessment of Fe-HA composite for temporary bone implants

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INTRODUCTION: Fe-5wt%HA composite has been shown a good *in vitro* cellular activity and evidence of a gradual *in vivo* degradation of its implants in the radial bone of sheep based on radiodensity image analysis [1,2]. The present work aims to analyze peri-implant osseointegration and osteoconduction of the composite compared to SS316L after their implantation in medio-proximal region of sheep radial bones.

METHODS: Five male sheep (age 10-12 month, weight 14-16 kg) were used. The implants were inserted into prepared defects where one sheep received one implant on each leg. The venous blood was collected and centrifuged at day 60. The Fe and Ca ions concentration were measured using an AA-7000 atomic absorption spectrophotometer (Shimadzu, Japan) and P concentration using a UV-200RS spectrometer (LW Scientific, USA) at 660 nm. The implants were viewed by VR-1020 X-ray radiography (Medical Corp, Japan) and then peri-implant gray scale was analyzed using Image-J software (NIH, USA) at day 60. The bone and implant were biopsied for histological examination at day 70. The tissues were then embedded in paraffin block and sliced at 5 µm thickness by microtome for hematoxylin and eosin stain.

RESULTS: Fig. 1 shows radiographic peri-implant images analysis for both implants at day 70 post-implantation. The peri-implant density of Fe-HA implant higher than SS316L was observed.

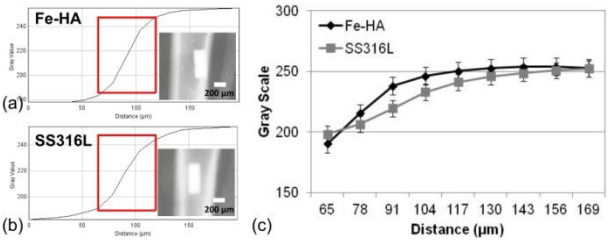

Fig. 1: Radiographic peri-implant at day 60. (a: Fe-HA),(b: SS316L), (c: plot profile peri-implant osteoconduction of both implants). Note: "red box" = region of interest.

Fig. 2 shows histological images of Fe-HA implant compared to SS316L at day 70 post-implantation. Fibrous tissue was observed on the Fe-HA implant whereas granular tissue was observed on SS316L surrounding tissues.

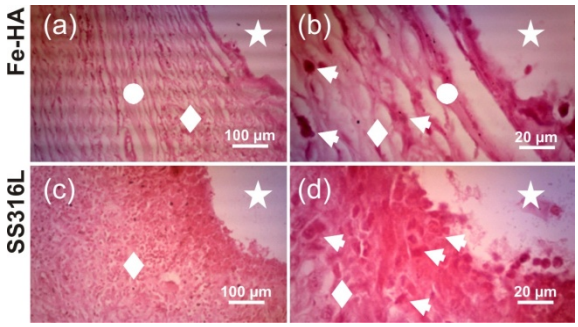

Fig. 2: Histological images around the implants at day 70. (a, b: Fe-HA), (c, d: SS316L). Note:"circle" = fibrous tissue, "diamond" = granular tissue, "star" = void after implant removal, "arrow" = giant cells.

Table 1 shows ion concentrations in the blood plasma. The result showed no different between both implants after day 60 post-implantation.

Table 1. Ion concentration in the blood plasma.

Group	Ion concentration (ppm)			
	Fe	Ca	P	Ca/P
Fe-HA	4.4±2.9	658.9±45.4	150.5±9.4	4.4±4.9
SS316L	5.2±2.8	635.4±24.4	191.1±37.9	3.3±0.7

DISCUSSION & CONCLUSIONS: Peri-implant assessment of Fe-HA composite was studied and compared to SS316L. The composite corresponded to induce fibrous tissue generation which covered the implant after day 70 post implantation indicating osseointegration process [3]. However, granulation process was observed on SS316L implant tissue. The formation of fibrous tissue caused Fe-HA to release ions locally. Addition of 5wt% HA to iron was increasing the peri-implant density which demonstrating osteoconduction process. In conclusion, incorporation of 5wt% HA to Fe enhanced the implant osseointegrative and osteoconductive to peri-implant tissue.

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Comparison of magnesium alloy and poly-L-lactide screws as degradable implant in canine fracture model

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INTRODUCTION: Non degradable steel- and titanium- based implants have been widely used for repairing of bone fracture. In order to reduce the invasive to the patients and treatment costs by re-operation, biodegradable materials are desired. In the present, these biodegradable implants are mainly composed of polymers as PLLA or PGA or their co-ploymers, PLGA [1]. The magnesium alloy is expected to novel biodegradable materials since the strength is three times of PLLA and its young's modulus close to the bone tissue [2-3]. Magnesium alloy degrades in vivo by corrosion reaction, and then, the hydrogen gas is generated. There are several methods of reducing the rate of evolution of hydrogen gas and the anodizing technique is one of most effective method for increasing the corrosion resistance [4]. A purpose of this study is to evaluate the biological response and effectiveness of biodegradable magnesium implants fabricated by anodizing WE43 for bone screw, comparing to monolithic WE43 metallic materials and PLLA screw.

METHODS: Nine 1-year-old beagle dogs weighing approximately 10 kg each were included in this study. Tested bone screws had a total length of 13mm and a shaft diameter of 2.6mm.The pitches of thread of the bone screw were 1mm. The tested magnesium implants were fabricated by anodizing of WE43. The anodic oxide film is about 10μm in thickness and contains Magnesium, oxygen and phosphorus. The used magnesium alloy in this study is Elektron SynerMag ® alloy supplied by Magnesium Elektron co. ltd., which contains magnesium (Mg) and yttrium (Y), rare earth (RE) element, and zirconium (Zr) and is named as a WE43. PLLA screws with the same dimension were used as a control. The bone osteotomy was created in the tibia of each dog. Each dog was then randomly divided into three groups on the basis of the screw material used to fix their osteotomy: monolithic WE43 (WE43 group), WE43 with anodizing (Anodized WE43 group) and Poly-L-Lactide screws (PLLA group). Two screws of these materials were fixed into each osteotomy and gypsums were carried out for 4 to 12 weeks. All 9 animals were euthanized after 4- and 12-week healing periods (n = 3 each).

RESULTS: In WE43 magnesium implant without anodizing, radiological and histological evaluation revealed that bone trabeculea around implanted WE43 decrease due to inflammatory response of hydrogen gas. However, there were no damages due to hydrogen gas and inflammatory response in a bone tissue around the tested anodizing WE43 magnesium implant. The results of the animal evaluation for one months, all PLLA (poly-L-lactic acid) implant (n=3) was broken but the tested magnesium implant did not break (n=3).

DISCUSSION & CONCLUSIONS: These results suggested that the anodized WE43 implant had enough strength to fix a bone fracture in load bearing site. Therefore, the magnesium implant was expected to as a new biodegradable material for bone screw or plate which can be applied to high load-bearing bone.

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The Evaluation of Histological Methods for Biodegradable Magnesium

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INTRODUCTION: The histological evaluation is an essential tool used in the field of orthopedics to examine the biocompatibility, bone fracture healing, and articular cartilage repair of implants. The examination of bone biopsies near the implanted sample provides valuable information on bone structure, remodelling, and turnover. Recently, there have been significant advances in development of biodegradable magnesium and its alloys as orthopedic implant materials. However, there are currently no standardized methods available in the literature to reflect on to perform the histological evaluation of the magnesium alloys and the unique corrosive characteristic of magnesium often hinders the accurate observation.

The purpose of this study was to evaluate the four most commonly used bone histological staining methods (Goldner's trichrome, Toludine blue, von Kossa with Gieson counterstain, Villanueva stain) to observe their effectiveness when used on magnesium.

METHODS: Four most commonly used staining methods for bone histologic analysis were performed using 0.25 mm thick and 1 mm long 99.9% pure magnesium samples to measure the weight loss after each staining procedure. Same standard protocol procedures were used for the slides made from in vivo test using New Zealand White Rabbits.

RESULTS: Goldner's trichrome stain showed most corrosion with 35.86 ± 4.15% weight loss. Toludine blue, Villanueva and von Kossa stained showed -1.32 ± 0.88%, -0.11 ± 0.81%, 1.79 ± 1.60% weight loss, respectably. Negative weight loss of Toludine blue and Villanueva stain is due to the stain residue left on top of the specimen. From the procedure of Goldner's trichrome method, Weigert's hematoxylin solution (mixture of hematoxylin A and B) caused most severe corrosion. After 10 minute of immersion, pure magnesium specimens lost 33.04 ± 2.14 % weight in hematoxylin A and 37.55 ± 3.02% weight in hematoxylin B.

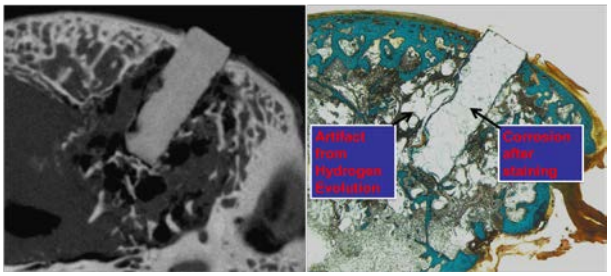

Fig. 1: Results of Goldner's trichrome stain

Histologic analysis of Mg alloys from in vivo test using Goldner's trichrome staining demonstrated corrosion of degrading implant sample after the stain was applied. Toludine blue staining and Villaneuva staining did not show the corrosion of specimen.

DISCUSSION & CONCLUSIONS: Goldner's trichrome staining method is the most commonly used staining procedure for the implant/bone evaluation. It provides accurate and precise analysis of new bone formation around the implanted samples made out of inert metals such as titanium and cobalt chrome alloy. However, severe corrosion of intact Mg alloy specimen was observed for Goldner's trichrome method and hydrogen gas generated from the corrosion hindered the accurate evaluation. This is due to the Ferric Chloride and Hydrochloric Acid in Goldner's trichrome (Weigert's hematoxylin solution), which are both extremely corrosive.

The result from this study suggests that the Toludine blue and Villaneuva staining methods, which contain basic solutions, are the ideal staining procedure to accurately evaluate the in vivo application of magnesium and its alloy.

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Biodegradable Mg-Cu alloy implants with antibacterial and osteostimulatory activity for the treatment of osteomyelitis: in vitro and in vivo evaluation

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INTRODUCTION: Despite advances in medical and surgical therapies, the treatment of osteomyelitis remains a clinical challenge, and implant removal is often necessary to achieve a cure. Accordingly, a novel therapeutic approach that permits implant retention is desirable. Therefore, the development of multifunctional bone implants for treating osteomyelitis and regenerating lost bone tissue, which may be a result of infection, is important. In the present study, implantable Mg-Cu alloys were designed to combine the favorable properties of magnesium, the antibacterial property of copper, and the osteostimulatory activity of Cu^{2+} ions.

METHODS: Five Mg-Cu alloys were fabricated, incorporating 0% (Mg0Cu), 0.05% (Mg0.05Cu), 0.1% (Mg0.1Cu), 0.25% (Mg0.25Cu), and 0.5% (Mg0.5Cu) copper, by weight. The morphologies and microstructures of the samples were investigated using field emission scanning electron microscopy (FESEM). Ionic extracts were prepared by soaking samples in alpha-minimum essential medium, osteogenic induction medium, and tryptic soy broth, according to ISO/EN 10993-5. The ionic concentrations of the Cu^{2+} and Mg^{2+} ions in the medium were measured by inductively coupled plasma mass spectroscopy. The *in vitro* effect of the released Cu^{2+} on the proliferation and osteogenic differentiation of human bone marrow stromal cells (hBMSCs) was determined using cell counting kit-8, alkaline phosphatase (ALP) activity, and reverse transcription-polymerase chain reaction analysis. The *in vitro* bactericidal property of the samples against *Staphylococcus epidermidis*, *Escherichia coli*, and *S. aureus* were determined using spread plates and crystal violet staining methods; bacterial morphology and adherence were observed by FESEM and confocal scanning laser microscopy (CLSM). *In vivo*, the tibial cavity of New Zealand White rabbits was injected with methicillin-resistant *S. aureus* strain to induce osteomyelitis, treated by debridement after 4 weeks, and implanted with Mg0.25Cu intra-medullary nails (Φ2.5 × 35 mm). The efficacy of the nails for treating osteomyelitis was evaluated using hematological, radiological, microbiological, and histological techniques. The amount of peri-implant new bone formation was evaluated using micro-computed tomography. The *in vivo* metabolic mechanisms of the Mg-Cu alloys were investigated using synchrotron radiation-based micro-computed tomography.

RESULTS: The results of the *in vitro* tests indicated that ionic extracts of the alloys significantly promoted osteogenic differentiation of hBMSCs by improving their bone-related gene expression (*ALP*, *OPN*, *OCN*). The CLSM and scanning electron microscope images showed that Mg0.1Cu and Mg0.25Cu extracts significantly inhibited bacterial adhesion and prevented biofilm formation by all bacterial strains. *In vivo*, the ideal therapeutic effect was observed in animals implanted with Mg0.25Cu intra-medullary nails, which resulted in significantly lower radiological and histological scores, a lower rate of positive *S. aureus* culture results, and excellent bone defect repair, without local or systemic side effects. No obvious Cu^{2+} or Mg^{2+} ion-complex deposition was found in the rabbit's organs or tissues.

DISCUSSION & CONCLUSIONS: Mg-Cu alloys demonstrate improved osteogenesis, and bactericidal properties, suggesting their potential use for the treatment of orthopedic infections.

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The effect of metallic magnesium degradation products on osteoclast-induced osteolysis and attenuation of NF-κB and NFATc1 signaling

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INTRODUCTION: Wear particle-induced aseptic prosthetic loosening is one of the most common reasons for total joint arthroplasty (TJA). Extensive bone destruction (osteolysis) by osteoclasts plays an important role in wear particle-induced peri-implant loosening. Thus, strategies for inhibiting osteoclast function may have therapeutic benefit for prosthetic loosening.

METHODS: In this study, we examined the effect of metallic magnesium degradation products (MDP) from magnesium (Mg), which has long been used in orthopedic implants with superior properties, in osteoclast formation and function and wear-particle-induced osteolysis. We mimicked the process of Mg degradation in vivo and obtained MDP by immersing pure Mg in culture medium. Firstly, in vitro we examined the MDP cytotoxicity via CCK8, flow cytometry, and colony assay. Then the effect of MDP on osteoclastogenesis, F-actin ring formation and bone resorption were examined via osteoclast-specific staining and SEM technique. Next a wear particle-induced osteolysis model was generated to examine the inhibitory effect of MDP in bone lesion in vivo via micro-CT and histological & histomorphometric analysis. Finally, molecular techniques were adopted to identify the potential mechanisms though which MDP inhibited osteoclast formation and function both in vitro and in vivo.

RESULTS: For the first time, we demonstrated that MDP suppresses osteoclast formation, polarization, and osteoclast bone resorption *in vitro*. An *in vivo* assay demonstrated that MDP attenuates wear particle-induced osteolysis. Furthermore, we found that MDP significantly inhibits nuclear factor-κB (NF-κB) activation by retarding inhibitor-κB degradation and subsequent NF-κB nuclear translocation. We also found that MDP attenuates the expression of NFATc1 at both the protein and mRNA levels. These results demonstrate that MDP has anti-osteoclast activity *in vitro* and prevents wear particle-induced osteolysis *in vivo*.

DISCUSSION & CONCLUSIONS: Collectively, our study suggests that metallic magnesium, one of the orthopedic implants with superior properties, has significant potential for the treatment of osteolysis-related diseases caused by excessive osteoclast formation and function.

*Contributed equally; #Co-corresponding authors.

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Ultra-pure magnesium alloys for use as biodegradable paediatric osteosynthetic material

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INTRODUCTION: Due to their excellent properties, magnesium (Mg) alloys are ideal candidates for the use in osteosynthesis [1]. Because a second operation for implant removal can be avoided they might be of special interest in paediatric orthopaedics. However, degradation of conventional Mg alloys is too fast and accompanied by the formation of high amounts of hydrogen gas [2]. To decelerate the corrosion rate, rare-earth elements or alloying elements of questionable toxicity have been frequently implemented, limiting magnesium's current application in medicine. This study investigates a new generation of ultra-high pure (XHP) magnesium alloys, containing only biocompatible elements such as zinc (Zn) and calcium (Ca).

METHODS: Two different ultra-high pure magnesium alloys (XHP-MgZn1Ca0.3 and XHP-MgZn1.5Ca0.25) were used [3]. Pins of 1.6 mm in diameter and 8 mm in length were implanted transcortically in 12 male Sprague-Dawley rats (n = 6 per group). The degradation rate, gas amount, implant-bone interface, and new bone growth were observed within a period of 6 months by means of continuous online µCT monitoring.

RESULTS: Both alloys show the desired low level of degradation. For the alloy XHP-MgZn1Ca0.3 no gas formation was clinically observable and the surrounding tissue resorbed the low amount of hydrogen gas formed during the slow degradation. The degradation rate was 'in equilibrium' with new bone growth and no adverse reaction of the surrounding bone was observed.

DISCUSSION & CONCLUSIONS: Ultra-high pure magnesium alloyed with well-balanced amounts of Zn and Ca exhibit a low degradation rate without the formation of hydrogen gas pockets. We attribute the slow degradation to the absence of micro-galvanic corrosion. This new generation of magnesium alloys is characterized by ultra-high purity and the addition of low amounts of Zn and Ca as alloying elements. These alloys fulfil all requirements for the clinical use and are promising as biodegradable osteosynthetic devices in paediatric orthopaedics.

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Increase of compact bone mass is the endpoint effect of MgO implants

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INTRODUCTION: In 1924, A. Zierold published a study "with the object to determine whether metal per se, when implanted in bone, exerts an influence other than that of any foreign body". Implanted Mg was shown to increase the amount of connective tissue (fibrosis) and callus-bone formed under the periosteum. Thus, since 90 years it has been known that Mg-implants are resorbed in the tissue, have both retarding and stimulating effect on the formation of bone during healing and induce fibrosis. The time dependence of bone healing at implants in rat tibia was described earlier. Callus bone was seen at 4 days after injury, and remodeling of the callus was seen in the second week. In the sham-operated controls the entire marrow cavity was cleared from callus bone within three weeks after surgery. An intense interest in Mg as a resorbable implant material is seen in recent years and several studies have been published on the effects of Mg on bone healing. In the present study, the effect of MgO on the healing of a drilled hole in rat tibia is followed over 3 weeks of implantation in order to find the endpoint effect of implanting MgO in bone marrow.

METHODS: Bone samples were fixed by cryosubstitution. Analysis of bone samples was made with by histology, TUNEL staining detecting apoptotic cells, Environmental SEM equipped with EDX and by TOF-SIMS.

RESULTS AND DISCUSSION: After 4 days of healing, an elevated level of Mg was detected in the bone marrow and the formation of callus bone was hampered in the Mg-treated animals. After 7 days, elevated levels of Mg were detected in the bone marrow of Mg-treated animals, and acellular bone was formed together with a marked fibrosis in the bone marrow cavity. The collagen-rich and cell-poor connective tissue did not contain elevated levels of inflammatory cells, suggesting chemical irritation caused the connective tissue reaction. After 14 days, there was no trace of the implanted Mg in the bone marrow space, and the healing of bone was in a later stage of remodeling in the Mg-treated animals, suggesting that Mg accelerated the remodeling process. After 21 days the bone was healed in both Mg-treated animals and sham-operated controls. The mass of compact bone was increased by 25% in the Mg-treated animals. The results indicate a profound effect of Mg on cell regulation of bone healing.

CONCLUSION: Implanting of MgO into the bone marrow cavity of rat tibia results in an early local irritation, accelerated remodeling and increased bone mass of compact bone

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Recommendation for modification of current cytotoxicity testing standards for biodegradable magnesium materials

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Abstract: As one of the most promising medical metal implants, magnesium (Mg) or its alloys have shown significant advantages over other candidates attributed to not only their excellent biodegradability (biocorrosion) suitable mechanical properties but also their osteopromotive effects for bone applications. Prior to approval mandated by the governmental regulatory body, the access to the medical market for Mg-based implants may require a series of testing for assurance of their safety and efficacy. Preclinical evaluations include both in vitro and in vivo biosafety tests and clinical phase 1 and 2 evaluations, and Phase 3 of multi-center randomized double blind and placebo-controlled clinical trials for Class III medical devices involving biomedical implants or devices. However, cytotoxicity tests based on current ISO 10993 standards for Class III medical implants may not apply to biodegradable metals due to substantial differences between in vitro and in vivo testing environments. Generally, more sophisticated compositions and circulation system in vivo would effectively retard the corrosion of degradable metals and excrete extra ions via surrounding tissue fluid and systemic blood circulation. Therefore, instead of a direct adoption, modification of ISO standards for in vitro cytotoxicity test is justified. The current paper recommended a 10 times dilution of extracts for in vitro cytotoxicity test for Mg or its alloy developed as potential orthopaedic implants based on literature review and our specifically designed in vitro and in vivo tests.

Keywords: Biometal, magnesium, biodegradability, cytotoxicity, ISO standards, modification