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Effect of equal channel angular pressing on *in vitro* degradation of LAE442 magnesium alloy

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ABSTRACT

Effect of processing by equal channel angular pressing (ECAP) on the degradation behaviour of extruded LAE442 magnesium alloy was investigated in a 0.1 M NaCl solution, Kirkland's biocorrosion medium (KBM) and Minimum Essential Medium (MEM), both with and without 10% of foetal bovine serum (FBS). Uniform degradation of as extruded and ECAP processed samples in NaCl solution was observed, nevertheless higher corrosion resistance was found in the latter material. The increase of corrosion resistance due to ECAP was observed also after 14-days immersion in all media used. Higher compactness of the corrosion layer formed on the samples after ECAP was responsible for the observed decrease of corrosion resistance, which was proven by scanning electron microscope investigation. Lower corrosion rate in media with FBS was observed and was explained by additional effect of protein incorporation on the corrosion layer stability. A cytotoxicity test using L929 cells was carried out to investigate possible effect of processing on the cell viability. Sufficient cytocompatibility of the extruded samples was observed with no adverse effects of the subsequent ECAP processing. In conclusion, this *in vitro* study proved that the degradation behaviour of the LAE442 alloy could be improved by subsequent ECAP processing and this material is a good candidate for future *in vivo* investigation.

1. Introduction

Magnesium alloys are nowadays intensively investigated as a potential material for degradable implants [1,2]. Until now, a high number of different Mg alloys have been investigated in different *in vitro* and *in vivo* studies [3], from which LAE442 alloy (Mg-4Li-4Al-2RE, wt%) is one of the most often investigated *in vivo* [4–11]. Relatively low and uniform degradation rate and no negative effect on the surrounding tissue and internal organs make this alloy one of the most suitable Mg alloys for orthopaedic applications. So far, all studies have been conducted on as-cast and extruded material. Comparative *in vivo* studies with as-cast LAE442 alloy showed superior performance compared to other alloys (AZ31, AZ91, WE43) after 18 weeks of implantation period [4,5]. However, gas cavities of evolved H_2 were observed in the surrounding tissue as a result of too rapid degradation rate. Improved *in vivo* degradation behaviour was found in the material after subsequent extrusion. A decrease of degradation rate and no presence of gas cavities after 12 weeks of implantation period were reported [6]. It was concluded that grain refinement and homogenization of alloying elements rich secondary phases were responsible for such improvement. Moreover, long-term *in vivo* studies showed superior degradation behaviour of extruded LAE442 alloy after 6 months [7] and 12 months [8] of implantation period in a rabbit model. Furthermore, the LAE442 alloy was found to have lower degradation rate than the WE43 alloy, which is currently considered as one of the most promising ones. The WE type alloy MAGNEZIX® is the first absorbable alloy which obtained the CE marking of Medical Devices for medical applications within Europe [12]. The significance of the LAE442 alloy in the orthopaedic implant research has been proven by a comprehensive *in vivo* study, in which degradation behaviour of intramedullary interlocked nailing system implanted in an adult sheep for 24-week period was investigated [9]. After a thorough investigation of implant volume development, *ex vivo* mechanical and histological examinations and elemental analyses of alloying elements in inner organs, the authors concluded that the extruded LAE442 alloy can be considered as a suitable degradable implant material. Additionally, the beneficial effect of grain refinement on the *in vivo* corrosion of the LAE442 alloy was demonstrated after implantation of a single extruded and a double extruded material into rabbits [10].

As mentioned, decrease of the degradation rate of the LAE442 alloy was observed after subsequent extrusion, which led to decrease of the grain size and more uniform distribution of secondary phases in the

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material. It was reported that even further improved grain refinement could be achieved in the LAE442 alloy after subsequent equal channel angular pressing (ECAP) [13]. Uniform distribution of equiaxed grains of ~1.5 μm in diameter and much more uniform distribution of secondary phases was observed after 12 passes through ECAP. ECAP was already found to be an effective method to increase corrosion resistance of different magnesium alloys [14–16]. Therefore, further increase of the corrosion resistance of the LAE442 alloy is expected when compared to the extruded samples. Moreover, substantial increase of the yield strength was observed in the fine-grained samples of the LAE442 alloy after ECAP [17]. Increase in mechanical strength could have beneficial effect on minimizing the implant size, which is important in particular applications. Furthermore, substantial grain refinement had a positive effect on the fatigue resistance, which was found to be possibly insufficient in the extruded samples of the LAE442 alloy [18]. Therefore, the ECAP processing of LAE442 alloy may enhance the degradable properties of the implant even further. To the best of our knowledge, the present study is the first one that aims to investigate the effect of ECAP on the *in vitro* degradation behaviour of the LAE442 magnesium alloy.

2. Experimental methods

2.1. Material

The investigated material was an extruded magnesium alloy LAE442 with the composition of 4.03 wt% Li – 3.56 wt% Al – 0.76 wt% La – 0.44 wt% Nd – 1.26 wt% Ce – 0.15 wt% Ca – 0.18 wt% Mn – < 0.0001 wt% Fe – <0.002 wt% Cu – <0.0002 Ni and balance Mg. Extrusion was performed at 350 °C with an extrusion ratio of 22. Billets with the dimensions of $10 \times 10 \times 100$ mm^3 were machined from the extruded bars and processed by ECAP. The ECAP processing direction was parallel to the extrusion direction. The processing was performed up to twelve passes (12P) following route B_C [19] in the temperature range of 185–230 °C and ram speed of 5–10 mm·min^{-1}. The angle Θ between two intersecting channels and the corner angle Ψ of the ECAP die were 90° and 0°, respectively. The samples of LAE442, which were extruded only, are designated Ex and the samples of LAE442, which were extruded and subsequently processed by ECAP, are designated 12P throughout the article.

2.2. Microstructure analysis

Microstructure of the specimens and corrosion layers after corrosion exposure were observed by scanning electron microscope (SEM) Zeiss AURIGA equipped by electron back scattered diffraction (EBSD) detector and energy-dispersive X-ray spectroscopy (EDS). Samples for microstructure observation were mechanically polished down to 50 nm alumina solution. Additional ion polishing was performed for EBSD specimens using Gatan PIPS™.

2.3. Corrosion in NaCl

Initial corrosion resistance of the studied samples was investigated by electrochemical impedance spectroscopy (EIS). The measurement was performed using three-electrode setup and controlled by the potentiostat AUTOLAB 120 N. Samples were cut perpendicular to the processing direction and exposed surface was ground with SiC1200 (15 μm) prior to each measurement. The measurement was performed in 0.1 M NaCl solution after 5 min of stabilization. EIS tests were executed at room temperature in the frequency range of 100 kHz-20 mHz with 10 mV amplitude with respect to the open circuit potential (OCP). Additional rotation of 1000 rpm was introduced to obtain better homogenization of the measurement. At least five measurements were performed for each sample/condition.

Hydrogen evolution was measured in 0.1 M NaCl solution at room temperature for one week of immersion. Samples with dimensions $8 \times 8 \times 3$ mm^3 were cut from the extruded and 12P bars. Subsequently they were ground (SiC1200, 15 μm) in ethanol in order to remove naturally occurring corrosion layer, measured and weighted.

2.4. Corrosion in biological media

Corrosion performance in biological media was investigated in Kirkland's biocorrosion medium (KBM, prepared according to [3] and buffered with $NaHCO_3$), in KBM + 10% FBS, in MEM (Sigma no. M0446) and MEM + 10% FBS. The ionic composition of both media in comparison with human plasma is given in Table 1. Samples with dimensions of $6 \times 6 \times 1.5$ mm^3 were cut from extruded and 12P bars. Before the tests, samples were ground (SiC1200, 15 μm) in ethanol in order to remove naturally occurring corrosion layer, measured, weighted and sonicated two times for 15 min in 96% ethanol. Three and two replicates of each sample type were used for corrosion rate determination and for corrosion layer examination, respectively. Samples were immersed into 40 ml (S/V ≈ 2.8 mm^2/ml, *i.e.* V/S = 36 ml/cm^2) of media in falcon tubes with vented caps (Corning) and incubated in 5% CO_2 atmosphere at 37 °C on an orbital shaker for 14 days.

After 14 days, the medium was removed and concentration of released Mg was measured using Atomic absorption spectrometer (AAS, Varian 220), while the pH of media was also recorded. Samples were immersed into a mixture of H_2CrO_4, $AgNO_3$ and $BaSO_4$ for 60 min at room temperature in order to remove the corrosion products. After drying, samples were weighted.

Corrosion rate (CR) was determined from the mass loss and was expressed in mg/cm^2/day. The corrosion rate was calculated using following Formula (1):

$$CR = \frac{\Delta m}{A \times t} \quad (1)$$

CR: corrosion rate (mg/cm^2/day), Δm: weight change in milligrams, A: surface area in cm^2, t: immersion time in days.

2.5. In vitro cytotoxicity testing

In vitro cytotoxicity of extracts (indirect test) was tested according to ISO 10993-5 standard. Samples ($6 \times 6 \times 1.5$ mm^3) were sterilized in ethanol for 2 h and then dried. Various surface-to-volume ratios and periods of incubation were used. In the first case, samples were immersed into 1 ml (S/V = 100 mm^2/ml) of cultivation media (MEM, Sigma M0446) without FBS, which is surface-to-volume ratio closer to that recommended in ISO 10993-12 standard. Samples were agitated on an orbital shaker (125 rpm) at 37 °C for 1 day. Thereafter, the extracts were centrifuged (5 min, 1500 ×*g*), supplemented with 10% FBS and immediately used for cytotoxicity testing. Three samples from each type (Ex and 12P) were used. Meanwhile, L929 cells (murine fibroblasts, ATCC® CCL-1™) were seeded into 96-well plates (100 μl/well) in the density of 1×10^5 cells/ml and incubated at 37 °C for 24 h in

Table 1
Ionic concentrations of KBM prepared according to [3] and of MEM + 10% FBS in comparison with human plasma.

Medium	KBM	MEM	Human plasma
Component	Concentration [mmol/l]		
Cl^-	102.5	126.9	103
Na^+	120.3	143.6	142
Ca^{2+}	2.5	1.8	2.5
K^+	5.1	5.4	5.0
Mg^{2+}	0.5	0.8	1.5
HPO_4^{2-}	0.9	1.0	1.0
SO_4^{2-}	0.5	0.8	0.5
HCO_3^{2-}	26.2	26.2	22–30
Glucose	5.0	5.6	5.0