Short communication

In vitro study on equal channel angular pressing AZ31 magnesium alloy with and without back pressure

X.N. Gu\textsuperscript{a}, N. Li\textsuperscript{a}, Y.F. Zheng\textsuperscript{a,\textdagger}, F. Kang\textsuperscript{b}, J.T. Wang\textsuperscript{b,\textdagger\textdaggerdouble}, Liquan Ruan\textsuperscript{c}

\textsuperscript{a} State Key Laboratory for Turbulence and Complex System and Department of Advanced Materials and Nanotechnology, College of Engineering, Peking University, Beijing 100871, China
\textsuperscript{b} School of Materials Science & Engineering, Nanjing University of Science & Technology, No. 200, Xiaolingwei, Nanjing 210094, China
\textsuperscript{c} Department of Mechanical Systems Engineering, Graduate School of Science and Technology, Kumamoto University, Karokami 2-39-1, Kumamoto-shi 860-8555, Japan

** Corresponding author. Tel.: +86 10 6276 7411; fax: +86 10 6276 7411.
\textdagger} Co-corresponding author.
\textdaggerdouble} E-mail addresses: yfzheng@pku.edu.cn (Y.F. Zheng), jtwang@mail.njust.edu.cn (J.T. Wang).

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\begin{abstract}
The equal channel angular pressing (ECAP) technique with and without back pressure (BP) was introduced in this paper to prepare biomedical AZ31 magnesium alloy, with the effect of pass number (from 1 to 4) on the corrosion properties as well as in vitro biocompatibility being investigated. The results indicated that ECAPed or BP-ECAPed AZ31 alloys exhibited similar corrosion rate to that of the as-extruded one, but the corrosion rate slightly increased after 1–2 passes ECAP or BP-ECAP and further decreased after 4-pass procedure. Additionally, severe local corrosion was observed for the 1–3 passes ECAPed or BP-ECAPed AZ31 alloy samples. Compared to the as-extruded AZ31 alloy, the samples after ECAP or BP-ECAP procedure showed much smaller sized corrosion pits on the surface after removing the corrosion product. The surface analysis after 20 days immersion in Hank’s solution revealed that the composition of the corrosion product consisted of C, O, Mg, P, Ca whereas only weak signal of Mg(OH)\textsubscript{2} could be detected beside the dominant \textgamma{(Mg)} peak by X-ray diffraction. The cytotoxicity results suggested that the multi-pass ECAPed or BP-ECAPed AZ31 alloy exhibited Grade I–II cytotoxicity according to ISO 10993-5: 1999.
\end{abstract}

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1. Introduction

Magnesium alloy is considered as a candidate for biodegradable implants due to close mechanical properties to bone, biocompatibility and biodegradability [1–3]. However, the practical usage of biomedical magnesium alloys faces some challenges, such as inherent low strength and fast corrosion rate. It has been reported that Mg–Zn alloy losses over 30\% bending strength with about 6\% weight loss [4] and pure Mg losses 70\% compressive yield strength with approximately 3\% weight loss [5]. That is to say, a high initial strength is recommended for magnesium alloys for clinical application. In addition, the fast corrosion of magnesium alloys, leading to mismatch of tissue healing rate and the gas cavity around the implants [4,6], also need to be improved.

The key issue is to improve the mechanical properties as well as the corrosion resistance of magnesium alloys at the same time. On this purpose, attempts have been proposed by means of alloying [2], heat treatment [7] and wrought process [2,4,6]. The wrought process has shown the benefit to improve both the strength and corrosion resistance effectively, without introducing additional alloying elements. Equal channel angular pressing (ECAP) is an effective processing technique to produce materials having homogeneous ultrafine grains with improved strength and exceptional superplastic capabilities [8]. Recent investigation has shown that the introduction of back pressure during the ECAP process (BP-ECAP) changes the mode of deformation to a simple shear, which enhances grain refinement from ECAP and improves the mechanical property considerably [9]. Our previous study [10] investigated the multi-pass ECAP AZ31 alloy with or without back pressure and the results indicated that the average grain size was reduced from 28 \textmu m to 8.5 \textmu m after 4-pass ECAP without back pressure, and the yield tensile strength (YS), ultimate tensile strength (UTS) and elongation (Elong.) changed from about (150 MPa, 230 MPa, 16.5\%) to (105 MPa, 280 MPa, 31\%). After 4-pass ECAP with back pressure, the average grain size further decreased to 1.7 \textmu m and the YS, UTS and Elong. values were improved to about (285 MPa, 430 MPa, 31\%). Therefore, multi-pass ECAP with back pressure seemed to be an effective technique for significantly improving the mechanical properties of magnesium alloys.

In this paper, AZ31 magnesium alloy was adopted as the material model and subjected to multi-pass ECAP with and without back pressure. The aim of this work was to evaluate the effect of
multi-pass (with or without back pressure) on corrosion resistance of magnesium alloys, thereafter the cytotoxicity was further evaluated.

2. Material and methods

The analyzed compositions of experimental AZ31 alloy material are 3.1165 wt.% Al, 1.1927 wt.% Zn, 0.3338 wt.% Mn, 0.0020 wt.% Fe, 0.0087 wt.% Si, 0.0016 wt.% Cu (balance Mg). The as-extruded rods (d/20 mm × 110 mm) were obtained with a reduction ratio of 16 at the extrusion rate of 24–30 mm/min extrusion rate at 300 °C. Then the extruded rods were subjected to 1–4 passes ECAP with and without back pressure following the procedure in our previous work [10]. The microstructures of the experimental samples were examined after etching process using 3% HNO3 solution by environmental scanning electron microscopy (ESEM, Quanta 200FEG).

The Hank's solution, dissolving NaCl 8 g/l, KCl 0.4 g/l, CaCl2 0.14 g/l, NaHCO3 0.35 g/l, MgSO4·7H2O 0.2 g/l, Na2HPO4·12H2O 0.12 g/l, KH2PO4 0.06 g/l in the distilled water, was used as electrolyte. The electrochemical corrosion tests were carried out using a conventional three-electrode chemical cell with platinum as a counter-electrode and saturated calomel electrode (SCE) as a reference electrode using an electro-chemical workstation (CHI600C, China). The working electrode is AZ31 alloy samples with the exposed area of 1 cm². The temperature was kept to be 37 ± 0.5 °C using a water bath. Potentiodynamic polarization tests were carried out at a scanning rate of 1 mV/s after immersing in Hank's solution for 10 min for stabilization. An average of three measurements was taken for each group.

The immersion test was carried out according to ASTM-G31-72 in Hank's solution at 37 ± 0.5 °C. Microstructural and compositional characterization of the corroded sample surface, before and after removing the corrosion products by CrO3 solution, was examined by ESEM equipped with the energy dispersive spectrum (EDS), X-ray diffractometer (XRD, Rigaku DMAX 2400) using a Cu Kα radiation was also employed. The amount of hydrogen generated from the samples was monitored using a calibrated burette according to the method described in reference [11] throughout the 20 days' immersion.

MG63 cells were adopted to evaluate the cytotoxicity of experimental samples by the indirect assay. The extracts were prepared using Dulbecco’s Modified Eagle's Medium (DMEM) serum free medium with the surface area of extraction medium ratio 1 ml/cm² in a humidified atmosphere with 5% CO2 at 37 °C for 72 h. The supernatant fluid was withdrawn after centrifugation and then refrigerated at 4 °C. MG63 cells were incubated in 96-well culture plates at 3 × 10³/mL for 24 h to allow attachment and the medium was then replaced with 100 μl extract. After 1, 2 and 3 days culture, MTT assay, based on the cleavage of the yellow tetrazolium salt MTT (thiazolybl tetrazolium bromide) into purple formazan by metabolically active cells, was adopted to evaluate the metabolic activity of MG63 cells. 10 μl MTT was added to each well cultured

![Fig. 1. Microstructures of (a) 1, (b) 4 passes ECAPed and (c) 1, (d) 4 passes BF-ECAPed AZ31 alloy.](image)
for 4 h and then 100 µl formazan solubilization solution was added incubating overnight. The spectrophotometrical absorbance of the samples was measured by microplate reader (Bio-RAD680) at 570 nm with a reference wavelength of 630 nm. The background with MTT results of extracts was subtracted according to reference [12]. The Mg, Al, Zn concentrations of the medium were also measured by the inductively coupled plasma atomic emission spectrometry (Leeman, Profile ICP-AES).

3. Results and discussion

Fig. 1 shows the microstructures of AZ31 alloy samples after 1 and 4 passes ECAP with or without back pressure. After 4 passes ECAP, the grains are greatly refined with an average grain size of 8.5 µm. For the sample after 1-pass BP-ECAP, the grain structure is inhomogeneous, with extremely fine grains of 1–2 µm as well as coarse grains of 5–10 µm because of the uncompleted dynamic recrystallization process. After 4-pass BP-ECAP, the microstructure becomes relatively homogeneous with an average grain size of 1.7 µm.

Fig. 2 shows the potentiodynamic polarization curves of multi-pass ECAPed AZ31 alloy with or without back pressure. For multi-pass ECAPed sample, the corrosion potential increases after 1–2 passes while decreases with increasing pass number. In contrast, the samples exhibit higher corrosion potential after multi-pass BP-ECAP. Song et al. [13] reported a large number of energetic crystalline defects generated during the ECAP process, and these sites favored the nucleation of MgO/Mg(OH)₂ protective films which resulted in the nobler corrosion potential. From Fig. 2(a), AZ31 alloy exhibits higher cathodic kinetics after 1–2 passes ECAP than the as-extruded one, and the cathodic kinetics are reduced after 3–4 passes. In addition, the corrosion current densities of multi-pass ECAPed samples indicate similar variation trend. In the case of BP-ECAPed AZ31 alloy, the corrosion current density increases after 1–3 passes while decreases significantly after 4-pass BP-ECAP. The 3BP-ECAPed sample shows highest corrosion current density, suggesting its highest corrosion rate. The current densities of 4-pass ECAP and BP-ECAP AZ31 samples are 16.52 µA/cm² and 25.37 µA/cm² in comparison with that of as-extruded AZ31 alloy 91.52 µA/cm².

Fig. 3 indicates the change of hydrogen evolution rate of multi-pass ECAPed and BP-ECAPed AZ31 alloy during the immersion period. Similarly with the electrochemical results, the AZ31 alloy samples show increased hydrogen evolution rate with 1–3 passes of ECAP and BP-ECAP process than that of the as-extruded state (as shown in inset in Fig. 3) after 20 days corrosion in Hank’s solution. However, we observed the reduction of hydrogen evolution rate for samples after 4 passes ECAP and BP-ECAP process, in comparison with the samples subjected deformation process with less pass number. After 20 days immersion, the corrosion product, composed of C, O, Mg, P, Ca, can be detected by EDS analysis. The XRD analysis identifies that the main phase of the corroded sample is still α(Mg) with some weak corrosion product peaks of Mg(OH)₂ visible, as shown in Fig. 4. It may due to the corrosion product formed on sample is too little to generate the X-ray signal to be detected, which also suggests the good corrosion resistance of ECAPed and BP-ECAPed AZ31 alloy sample.

Many previous works reported that fine-grained magnesium alloys, such as AZ31 [14,15], AZ91 [16,17] and Mg–Ca alloys [6], could exhibit a significantly reduced corrosion rate compared to the coarse-grained one. On the basis of this assumption, the further decreased corrosion rate is expected for AZ31 alloy after grain refinement by enough pass number of ECAP and BP-ECAP processing. It is interesting to find slightly enhanced corrosion after further grain refinement by 1–3 passes ECAP or BP-ECAP techniques. One possible explanation is likely attributed to the cumulative residual strains during deformation leading to more energy restored in the deformed grains and grain boundaries [13], which results in higher sensitivity of deformed grains and grain boundaries to the aggressive environment. Similar results, showing higher corrosion rate of ECAPed pure Mg [13] and AZ91 [18] than the as-cast state, were also reported. Another explanation might

Fig. 2. The potentiodynamic polarization curves of multi-pass (a) ECAPed and (b) BP-ECAPed AZ31 alloy in comparison with as-extruded AZ31 alloy in Hank’s solution.

Fig. 3. The change of hydrogen evolution rate of multi-pass ECAPed and BP-ECAPed AZ31 alloy during the immersion period in Hank’s solution.
be related to the gradual transformation of subgrain-boundaries from low angle type to high angle ones after ECAP by absorbing dislocations generated during processing and high angle grain boundaries are reported to be more susceptible to chemical attack with higher grain boundary energies [19,20]. Due to the large deformation amount for BP-ECAP techniques, a higher number of energetic crystalline defects (i.e. dislocations, grain boundaries) are expected to restore in the grains and grain boundaries of AZ31 alloy after 3 BP-ECAP process. Therefore, the higher corrosion activation induced by higher number of crystalline defects [13] than other samples might be responsible for poorest corrosion resistance of 3BP-ECAP sample. With further increasing the pass number (4-pass ECAP or BP-ECAP process), the corrosion rate of sample decreases compared to those subjected with less pass number. It may because that the inhomogeneous large sized grains (Fig. 1(a) and (c)), restored a number of strains and dislocations inside of the grains during the first several passes deformation, will recrystallize when subjected with more passes deformation and thus may release the residual internal strain and decrease the intragranular dislocation density [21]. Therefore, it will not be difficult to comprehend the increased corrosion resistance of 4-pass ECAPed and BP-ECAPed AZ31 alloy samples with less energetic crystalline defects.

Fig. 5 shows the corroded surface morphologies of multi-pass ECAPed and BP-ECAPed AZ31 alloy for 20 days immersion after
removing the corrosion product. Severe local corrosion pits can be seen on all the corroded surfaces while locally the mechanical polish scratch is still visible after 20 days' corrosion (Fig. 6). In addition, fewer pits are observed for 4-pass ECAPed and BP-ECAPed AZ31 alloy. As seen from the enlarged images (Fig. 6), 4-pass BP-ECAPed AZ31 exhibits much smaller pitting holes (1–2 μm) on the surface in comparison to the bigger pitting holes with size (5–10 μm) for as-extruded AZ31 alloy. Therefore, a relative uniform corrosion tendency with smaller pitting holes is expected after subjecting the ECAP technique, given the fine grain size minimal the distance between galvanic couple.

Fig. 7 shows the cytotoxicity of as-extruded AZ31 alloy and multi-pass ECAPed and BP-ECAPed AZ31 alloy. The ICP results indicate that Mg concentration in different extracts varies in a narrow range 4.41–4.93 mM, as well as the Al and Zn concentration falls in 0.37–0.49 mM and 11.01–13.03 μM, except for the 3-pass BP-ECAPed AZ31 alloy exhibiting a higher Mg (5.71 ± 0.67 mM) and Al (0.70 ± 0.09 mM) concentration. The cytotoxicity result indicates that as-extruded AZ31 alloy and multi-pass ECAPed and BP-ECAPed AZ31 alloys exhibit acceptable toxicity to MG63 cells with grade I toxicity, which is comparable as other published Mg alloys, such as Mg–Ca [6] and Mg–Zn [4], showing good biocompatibility from the in vivo study. However, 3-pass BP-ECAPed AZ31 alloy indicates grade II toxicity, which may be attributed to the higher Mg and Al concentration leading to the osmolarity shock to the cells [22]. On the basis of the obtained results, the ECAP with back pressure procedure may be recommended since it can improve the strength and ductility of magnesium alloys considerably. The pass number for the ECAP with back pressure process should be optimized for different magnesium alloys to screen appropriate corrosion rate for future biomedical application.

4. Conclusions

In this paper, the corrosion behavior of magnesium alloy had been studied in respect to the effect of pass number for ECAP with or without back pressure using AZ31 alloy model. The multi-pass ECAPed and BP-ECAPed AZ31 alloy exhibited close corrosion rate to the as-extruded one. Small increment of corrosion rate as well as the severe local corrosion could be found after 1–2 passes ECAP with or without back pressure during 20 days’ immersion in Hank’s solution. After 4-pass ECAP and BP-ECAP process, AZ31 alloy indicated reduced corrosion rate with less and smaller corrosion pits on the sample surface. Besides 3BP-ECAPed one, other multi-pass ECAPed and BP-ECAPed AZ31 alloy indicated Grade I toxicity to MG63 cells. Therefore, the ECAP with back pressure seemed to be an effective procedure to improve the mechanical property of magnesium alloy without significantly reducing the corrosion resistance for the biomedical application of magnesium alloys.

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