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In vitro and *in vivo* studies of biodegradable fine grained AZ31 magnesium alloy produced by equal channel angular pressing

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ABSTRACT

The objective of the present work is to investigate the role of different grain sizes produced by equal channel angular pressing (ECAP) on the degradation behavior of magnesium alloy using *in vitro* and *in vivo* studies. Commercially available AZ31 magnesium alloy was selected and processed by ECAP at 300 °C for up to four passes using route B_c. Grain refinement from a starting size of 46 μm to a grain size distribution of 1–5 μm was successfully achieved after the 4th pass. Wettability of ECAPed samples assessed by contact angle measurements was found to increase due to the fine grain structure. *In vitro* degradation and bioactivity of the samples studied by immersing in super saturated simulated body fluid (SBF 5×) showed rapid mineralization within 24 h due to the increased wettability in fine grained AZ31 Mg alloy. Corrosion behavior of the samples assessed by weight loss and electrochemical tests conducted in SBF 5× clearly showed the prominent role of enhanced mineral deposition on ECAPed AZ31 Mg in controlling the abnormal degradation. Cytotoxicity studies by MTT colorimetric assay showed that all the samples are viable. Additionally, cell adhesion was excellent for ECAPed samples particularly for the 3rd and 4th pass samples. *In vivo* experiments conducted using New Zealand White rabbits clearly showed lower degradation rate for ECAPed sample compared with annealed AZ31 Mg alloy and all the samples showed biocompatibility and no health abnormalities were noticed in the animals after 60 days of *in vivo* studies. These results suggest that the grain size plays an important role in degradation management of magnesium alloys and ECAP technique can be adopted to achieve fine grain structures for developing degradable magnesium alloys for biomedical applications.

1. Introduction

Research on magnesium based materials as potential candidates for degradable temporary implants has been gaining wide popularity because of their mechanical properties which are close to that of natural bone compared to other metals, their good biocompatibility, non-toxicity and biodegradability [1–5]. However, rapid degradation in physiological environment with the evolution of hydrogen gas restricts its wide usage as a biomaterial [6–8]. Many new magnesium alloys and composites have been produced and evaluated for biomaterial applications [3,9–14]. Also, different coatings have been employed on magnesium alloys in order to improve the corrosion resistance [15–18]. These technological advancements help in addressing the issue of uncontrolled degradation of magnesium based biodegradable implants targeted for temporary applications where the second surgical procedure to remove the implant after the tissue is sufficiently healed can be completely avoided.

It has been well established that the microstructure plays an important role in altering mechanical, chemical, wear, electrical and corrosion properties [19–23]. When a polycrystalline metal undergoes grain refinement, the grain boundary area is enormously multiplied and the fraction of available grain boundary area in the bulk metal is increased. Generally, grain boundaries are high energy sites and therefore, material properties will be affected to a great extent as the fraction of grain boundary is increased. Properties which are related to surface energy undergo a significant change with grain refinement. In biomedical field, the promising role of micro/nano featuring of materials and substrates with high surface energies in enhancing the implant–tissue interactions has been clearly demonstrated [24–27].

Grain refinement was achieved in magnesium and its alloys by different processing routes and interestingly many alloys showed improved corrosion resistance [28–36] and a few alloys exhibited decreased corrosion resistance [34–37]. From these early reports, it is clearly observed that along with the grain size, other microstructural

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features like twins, stacking faults, dislocations and distribution of secondary phase particles play an important role in influencing the corrosion behavior of magnesium alloys. However, information regarding the effect of reducing the grain size on biomineralization and the influence of improved bioactivity on the degradation behavior of magnesium based materials, especially during the initial hours of degradation, is not well established.

Therefore, the objective of the present study is to investigate the role of grain size produced by equal channel angular pressing (ECAP) on the bioactivity and degradation of commercially available AZ31 Mg alloy by conducting *in vitro* and *in vivo* experiments. ECAP, a severe plastic deformation (SPD) technique used to develop nano/ultra fine grained metals was adopted and fine grain structured AZ31 Mg alloy was successfully produced. The effect of grain size on the wettability, a significant property which influences the tissue–implant interactions and healing rate was investigated. *In vitro* bioactivity and degradation behavior were studied by immersion tests and *in vivo* experiments were carried out using rabbit animal model.

2. Materials and methods

2.1. Experimental details

Commercially available AZ31 magnesium alloy (Exclusive Magnesium, Hyderabad, India) of chemical composition 2.9%Al, 0.88%Zn, 0.001%Fe, 0.02%Mn and remaining being Mg (in at.%) was annealed at 340 °C for 30 min and furnace cooled. Then the samples were heated to 300 °C and ECAP was carried out up to four passes using B_c route. The principle behind grain refinement in ECAP has been explained elsewhere [38]. Molybdenum disulfide (MoS_2) was used as the lubricant to reduce the friction during the process. After each pass, the specimen ends were made straight and faced using a lathe and the sample surface was polished. The specimens were then subjected to further pressings. The die used for ECAP consisted of two channels 10 mm in diameter intersecting at a die angle of 120° as shown in Fig. 1. Samples of diameter 10 mm and length 100 mm were used in the present study. The height to diameter ratio of the punches used to press the samples was kept as 3 to avoid buckling. Hence, four separate punches were used to complete the pressing. The samples were coded as AS AZ31, ECAP1st, ECAP2nd, ECAP3rd and ECAP4th for annealed AZ31 magnesium alloy and after ECAP first, second, third and fourth passes respectively.

2.2. Characterizations

Microstructural observations were carried out using optical microscopy and transmission electron microscopy (TEM). Samples were cut from the center of the ECAPed rods transverse to the pressing direction and metallographically ground using emery papers up to 2000 grade and cleaned with ethanol. Further, the samples were polished using diamond paste (1–3 μm grit size) with the help of disk polishing machine (Binpol — VTD, Chennai Metco, India). The polished samples were ultrasonically cleaned using ethanol and etched using picric acid reagent. For TEM observations (TEM, Philips CM12, Holland, operated at 120 kV), thin disks were cut from the center of the samples and manually polished up to a thickness of 100 μm. Then twin jet polishing was carried out using an electrolyte solution comprising of 1% perchloric acid and 99% ethanol. Wettability of the samples was studied by contact angle measurements using water as the solvent. Measurements were obtained at five different locations on each sample.

2.3. In vitro bioactivity

Bioactivity studies were carried out by immersing the samples in super saturated simulated body fluid (SBF 5×) kept at a constant temperature (37 °C). The ion concentrations of SBF are similar to human blood plasma and the preparation methodology was as reported by Kokubo et al. [39]. The ion concentrations in SBF 5× are shown in Table 1. Super saturated concentrations have been used in the present study to accelerate the mineralization and to quickly assess the role of deposited mineral phases on the degradation behavior of the samples. The study was carried out for 24, 48 and 72 h. The ratio of the apparent surface area of the sample to the SBF volume was approximately maintained as 1:20. The samples were removed from SBF at different intervals of time, gently washed in de-ionized water and subjected to different characterizations. The phase analysis was done by X-ray powder diffraction (XRD, D8 DISCOVER, Bruker, USA) with Cu Kα radiation ($\lambda = 1.54$ Å) at a scanning rate of 1 step/s and step size of 0.1°/step. The surface morphology of the samples after immersion was observed using scanning electron microscope (SEM, Quanta 200, FEI, Netherlands) operated at 30 kV. The elemental composition of the mineral phases deposited on the samples after immersion was investigated by energy dispersive X-ray (EDS) analysis.

2.4. In vitro degradation

2.4.1. Weight loss test

Degradation behavior of the samples by weight loss method was studied by immersing the samples in SBF 5× for 72 h. The samples were immersed in a volume of 500 ml of SBF 5× kept at a constant temperature (37 °C). Then the samples were removed from SBF 5× and the corrosion products were removed by immersing in a boiling solution of chromic acid (180 g/1 l of de-ionized water) for 10 min. The samples were then dried and the weight loss was measured by using the

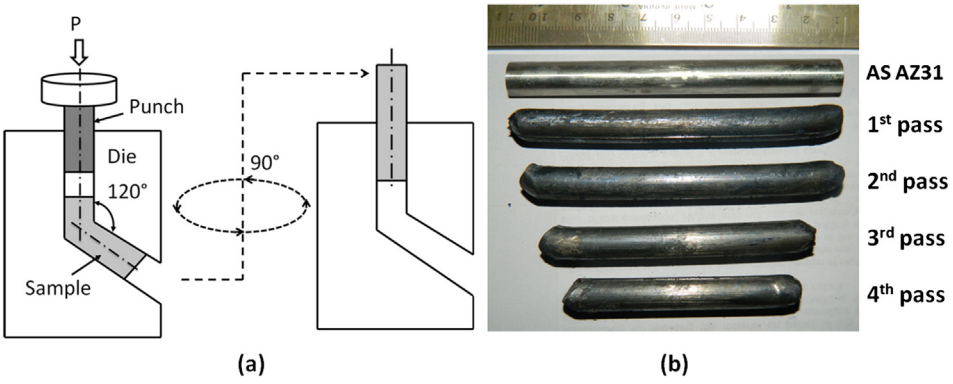

Fig. 1. a) Schematic representation of ECAP route B_c and b) photographs of the samples.