

Introducing an ultralight, high-strength, biodegradable Mg-4Li-1Ca alloy

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Mg-4Li-1Ca ternary alloy produced by melting from two binary master alloys was subjected to two-step rolling undergoing 60% thickness reduction in each. The density of the wrought alloy was found to be 1.56 ± 0.3 gm/cm³—lower than the density of any commercial Mg alloys. Rolling resulted in a remarkable improvement in tensile strength (250 MPa) owing to substantial microstructure refinement and a rather uniform dispersion of second phase particles. Biodegradation behaviour in simulated physiological environments confirmed the formation of a hydroxyapatite layer ensuring good biocompatibility of the alloy. The live/dead staining results with osteoblast cells showed a substantial fraction of live cells upon seven days of culture, which indicates excellent cytocompatibility of the alloy. This was further supported by the scanning electron microscopy evidence. We conclude that the new Mg-4Li-1Ca alloy is a promising material for medical implants possessing a favourable combination of properties, including low density, high strength, and promising cell response.

Keywords: Mg-4Li-1Ca alloy, biodegradation, implant application, cytocompatibility, live-dead staining, two step rolling

1. Introduction

Developing new biodegradable Mg alloys for implantology based on binary alloys (e.g. Mg-Al, Mg-Rare Earths (RE) and Mg-Ca), ternary alloys (such as Mg-Al-Ca and Mg-Zn-Ca), or other more complex Mg-based systems is becoming a subject of great interest [1–3]. Mg-Al-Zn and Mg-RE alloys are heading towards commercialisation as bioresorbable implant materials. However, they are heavier than Mg. In addition RE and especially Al content is a matter of concern for biodegradable implant applications. Among the candidate alloys, Mg-Ca alloys appear to have a great potential as far as good biocompatibility and strength are concerned, in spite of their poor formability [4–6]. It has also been reported in literature that addition of Li helps in reducing the density and improving the ductility of Mg. Lithium has long been applied in healthcare and is considered to be nontoxic if its concentration in bodily fluids does not exceed 75 ppm [7].

The addition of either Ca or Li to Mg taken in isolation has some limitations. Calcium alone makes the alloy highly brittle, and its corrosion resistance decreases with increasing Ca content. Alloying with lithium also has an adverse effect on corrosion properties. Furthermore, an increase of Li content makes the microstructure highly unstable, thereby resulting in inferior mechanical properties [8, 9]. A detailed study of binary Mg-Li, Mg-Ca and Li-Ca phase diagrams suggests that in the ternary Mg-Ca-Li system calcium will form a eutectic due to a high driving force for the formation of Mg-Ca compounds rather than forming a Li-Ca eutectic [8]. It is expected that this will be useful for the improvement of the mechanical properties of the system as well as governing the degradation mechanism [8, 9].

Song and Krol reported a substantial improvement in the mechanical properties by cold rolling of Mg-11Li- x Ca ($x = 5$ –15 wt %) alloys [10]. Recently, the mechanical properties of the newly developed tertiary alloy Mg-4Li-1Ca (henceforth referred to as LC41) was reported [8, 10, 11]. It was shown that the microstructure of the alloy produced by rolling with and without subsequent annealing has a strong effect on the mechanical and biocorrosion pro-

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erties. It was concluded that this tertiary alloy exhibits a very favourable combination of specific strength, formability and biocorrosion resistance [8, 10, 11]. Here we present a brief summary of the mechanical properties, biodegradation behaviour, and cytocompatibility of the new Mg-4Li-1Ca alloy, which can be considered as a promising candidate for structural and implant applications.

2. Experimental methods

Samples with the dimensions of $101 \times 25 \times 4$ mm³ were cut from the ingot and subjected to homogenization treatment at 350°C for 2 h prior to rolling. The detailed rolling schemes employed are given in Table 1. Samples cut from the rolled plate were mechanically polished up to 0.25 µm diamond paste and etched with 10% Nital solution (10% HNO₃ and 90% Ethanol) for 25–30 s. Microstructure was observed by optical microscopy (OM) and scanning electron microscopy (SEM). Tensile specimens with a gauge length of 16 mm and width of 6 mm were deformed at a constant nominal strain rate of 10^{-4} s⁻¹ in an INSTRON testing machine at ambient temperature.

In order to investigate biodegradation behaviour, samples with an exposure area of 10 mm × 10 mm were subjected to immersion tests in a simulated body fluid (SBF, Kokubo's solution, all raw chemicals supplied by Sigma-Aldrich) as well as in Earle's Balanced Salt Solution (EBSS supplied by Sigma-Aldrich) for 7 days at a starting pH of 7.2. The surface morphology after immersion was characterized by SEM, and the formation of corrosion products was analysed by Energy Dispersive Spectroscopy (EDS) and X-ray Diffraction (XRD). After immersion tests, the release of Li into the SBF was measured by Plasma Atomic Emission Spectroscopy (ICP-AES).

Samples were sterilised ultrasonically for 20 min in 70% ethanol (Sigma-Aldrich Chemie GmbH, Munich, Germany). Prior to cell tests, samples were incubated overnight in cell specific complete medium. Human bone derived cells (HBDC; i.e., osteoblast primary cell model) isolation was approved by the local ethics committee and adapted from Gartland et al. [12]. HBDC were cultured in Dulbecco's

Modified Eagle's Medium (DMEM) supplemented with 10% foetal bovine serum (FBS, Merck, Darmstadt, Germany), 1% penicillin, and 100 mg mL⁻¹ streptomycin (Life Sciences, Karlsruhe, Germany) in an incubator under physiological conditions (5% CO₂, 20% O₂, 95% relative humidity, 37°C). 50000 cells in 50 µL medium were seeded on samples in 12-well plates and let adhere onto the surface for 30 min. Thereafter 3 mL of medium were added to each well.

Cell viability was assessed via Live/Dead Viability/Cytotoxicity Kit (Life Technologies, Darmstadt, Germany), a two-color fluorescence assay. After removing the cell culture medium, samples were washed with phosphate buffer solution (PBS, 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na₂HPO₄, 1.4 mM KH₂PO₄, all chemicals from Sigma-Aldrich Chemie GmbH, Munich, Germany), then transferred to a new 12-well-plate and covered with the staining solution (2 µl Calcein AM and 5 µL ethidiumhomidimer-1 in 5 ml PBS) and incubated for 20 min. Afterwards the staining solution was replaced by PBS and samples were directly examined with a fluorescent microscope (Eclipse Ti-S, Nikon, Düsseldorf, Germany). Living cells can be detected by using the FITC-filter (Ex: 465–495 nm, Em: 515–555 nm, Mirror at 505 nm) as green areas, dead cells can be observed by using the Texas Red-filter (Ex: 540–580 nm, Em: 600–660 nm, Mirror at 595 nm) as red areas. The overlaying image was merged by the microscope software.

After being washed in PBS, samples were critical point dried previous to SEM evaluation (Auriga; Carl Zeiss, Jena, Germany). In brief, after a glutaraldehyde (Sigma-Aldrich Chemie GmbH, Munich, Germany) fixation step, carriers were stained in osmium tetroxide (Sigma-Aldrich Chemie GmbH, Munich, Germany) prior to an alcoholic dehydration row. Subsequently, samples were critical point dried in 2-propanol (Sigma-Aldrich Chemie GmbH, Munich, Germany) to preserve cell morphology for observation in a Leica EM CPD300 (Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany). Cells on carriers were then visualised by low voltage mode in charge contrast, using the SEM (Auriga; Carl Zeiss, Jena, Germany) in lens detector.

3. Results and discussion

3.1. Microstructural evolution and mechanical properties in new LC41 alloy

Microstructures of the as-cast and homogenised (labelled "homo") LC41 alloy are shown in Figs. 1a, b, respectively. The light region shows the α-Mg rich phase, with the dark areas within the grains and at the grain boundaries corresponding to the eutectic (α + Mg₂Ca) phase [6, 8]. Especially in the homogenised condition, the morphology of the eutectic is continuous and elongated (α/Mg₂Ca lamellar) at dendrite boundaries, whereas that located within the dendrites are dumbbell-shaped [6], as seen in the SEM image in Fig. 1b.

Table 1. Detailed rolling schemes used for the processing of LC41 alloy

Rolling scheme	Details
R1	Homogenized samples were rolled at 300°C to 60% thickness reduction in 12 passes with intermediate 10 min annealing at 300°C between the passes
R2	Homogenized samples were rolled to total 60% thickness reduction by first subjecting them to initial rolling at 350°C from 4 mm to 2 mm thickness in 10 passes followed by air cooling, and further rolling at 200°C in 2 passes to final thickness of 1.6 mm

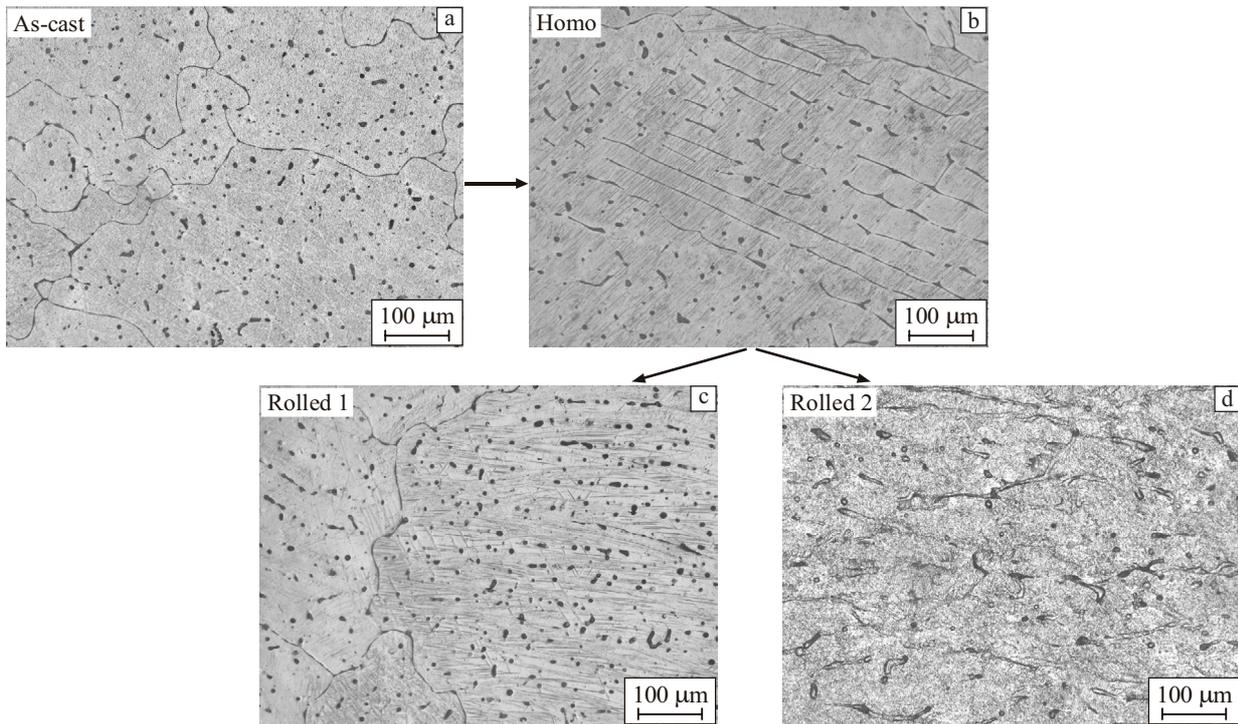


Fig. 1. Optical micrographs elaborating microstructure evolution in LC41 alloy.

Figures 1c and 1d show the microstructures of LC41 alloy evolved upon R1 and R2, respectively. In case R1, the deformed microstructure showed an appreciable volume fraction of deformation twins [8], whereas the R2-processed material exhibited extraordinary grain refinement with average grain size of 300 nm. Overall, rolling resulted in pronounced improvement in the mechanical properties over those of the as-cast alloy, as seen from Table 2.

Examination of the microstructure confirmed that the addition of Ca to Mg-Li leads to the formation of a eutectic containing Mg_2Ca particles well distributed at the boundaries depending on the Ca content. These Mg_2Ca particles restrict grain growth as reported by Zeng et al. [13] for a different Mg-Ca-Li alloy (hot extruded Mg-9.27Li-0.88Ca). In the present case R1-processed alloy showed the occurrence of a significant number of $\{10\bar{1}1\} - \{10\bar{1}2\}$ double twins. In the R2-processed alloy, an ultrafine equiaxed grain structure was observed. This highly desirable grain refinement is attributed to a greater driving force for dynamic

recrystallization during rolling at 350°C used in the first step of the R2 schedule. The second step, i.e. rolling at 200°C, led to severe deformation without much recrystallization or grain coarsening thereby resulting in a very high strength of 245 MPa (to be compared to that of 208 MPa for R1 regime) [14].

3.2. Biodegradation and cytocompatibility of LC41 alloy

The immersion of LC41 specimens in the physiological medium resulted in their rapid degradation, which led to formation of various corrosion products. Figure 2a shows SEM images of the specimen surface after immersion in EBSS for 7 days. Owing to the dehydration reaction occurring during biodegradation [6], cracks on the corroded surfaces were observed. White Ca and P rich compounds observed on the surface (cf. Figs. 2b and 2c) were identified as hydroxyapatite (HA). Also confirmed by XRD (Fig. 2c) were the metastable octacalciumphosphate (OCP) and magnesium hydroxide phases. The process of degradation and formation of these products is very complex and the driving force for the formation of each product is highly dependent on the local pH value of the environment [6, 15]. A possible scenario of the formation of the biocorrosion products on the surface of LC41 was outlined in our earlier publication [6]. The formation of a protective HA layer is an indication of a good biocompatibility of the alloy in simulated body environment.

HBDC were directly cultured onto the sample surface for 14 days and their viability assessed by live/dead staining.

Table 2. Mechanical properties of LC41 alloy for different processing histories

Condition	UTS, MPa	YS, MPa	Hardness, VPN
As-cast	123.6	70.6	58
Homo	104.0	57.0	49
R1	208.0	190.0	67
R2	245.0	210.0	75

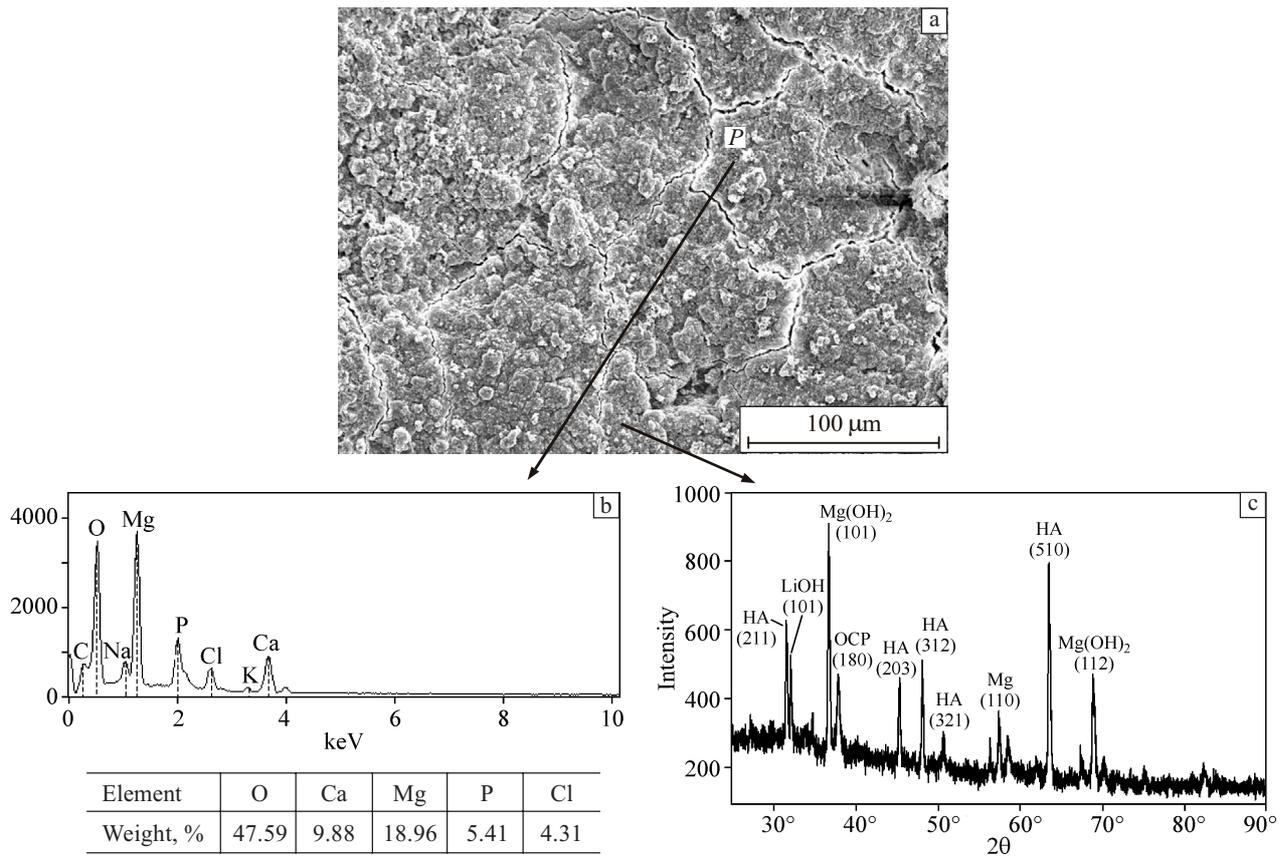


Fig. 2. Secondary electron image showing surface morphology of LC41 alloy upon immersion in EBSS for 7 days (a), EDAX scan at point *P* (b), XRD pattern (c).

As can be observed in Fig. 3, many cells are live while a few are dead. HBDC are widely spread on the material surface (Fig. 3a). Furthermore, HBDC exhibit flattened, elongated morphology, characteristic of osteoblast cells (Fig. 3b). Similar observations can be done on SEM pictures (Fig. 4).

Cytocompatibility assessed with regard to the osteoblastic HBDC as tested by live/dead staining also showed

good cell viability (green cells in Fig. 3). An SEM study with the same primary cells indicated good attachment of the cells to the material. Moreover, well-spread cells having a healthy morphology throughout the specimen surface were observed upon 14 days culture [12].

These preliminary studies suggest that the newly developed Mg-4Li-1Ca alloy exhibits promising cytocompati-

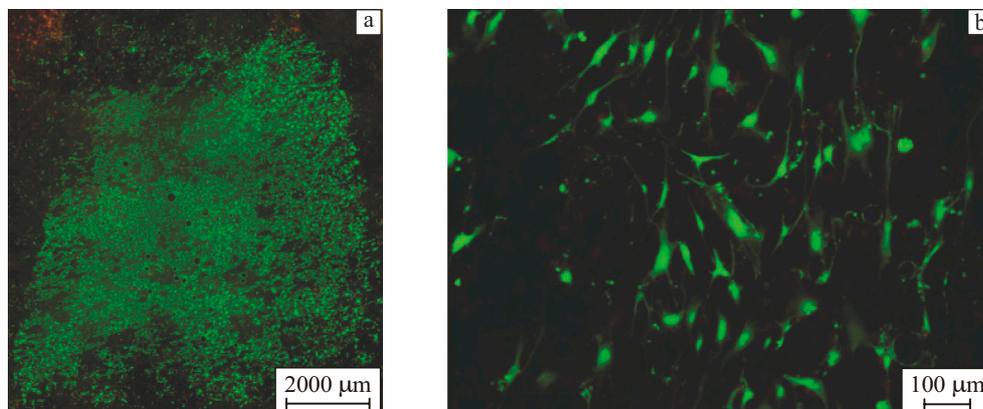


Fig. 3. Live/dead staining images with osteoblast primary cells (HBDC) on LC41: overall surface showing live cells (a) and a magnified view of live cells (b).

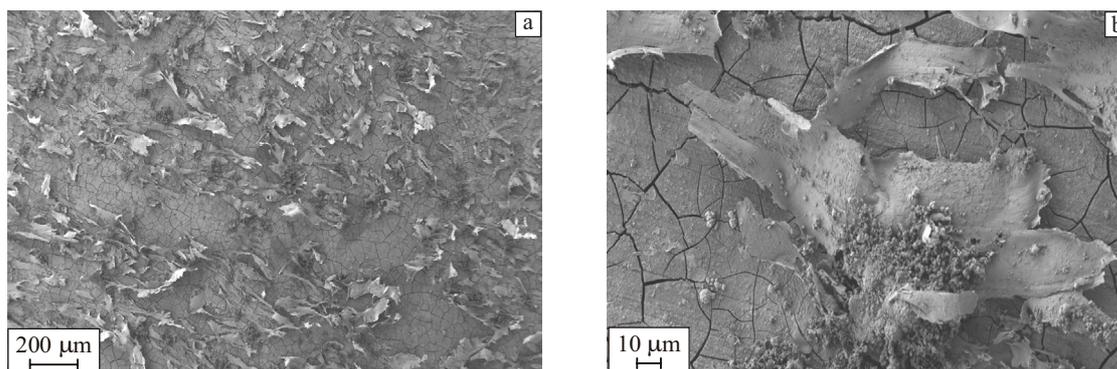


Fig. 4. Secondary electron image showing overall osteoblast cell attachment on LC41 alloy surface (a), magnified view of an osteoblast cell attached to the surface (b).

lity properties. This material could be an ideal replacement for the existing biodegradable implant materials because it possesses low density and high strength without compromising cytocompatibility [4, 12].

4. Conclusions

Our attempt to develop a new ultralight alloy outperforming most magnesium-based alloys in terms of a combination of density, strength, biodegradability and cytocompatibility proved to be successful. The LC41 alloy possesses an ultimate strength as high as 245 MPa and a density as low as 1.59 g/cm³. Besides, it forms a protective layer of hydroxyapatite in a simulated body environment. Osteoblast cell response to this alloy is also favourable. Thus, this newly developed alloy can be considered as a promising competitor of Mg-based alloys for biodegradable implant applications.

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