Effect of grain refinement on biodegradation and bio-mineralization of low calcium containing Mg–Ca alloy

R Shishir¹, Shebeer A Rahim², and T Hanas¹,²

¹ Nanomaterials and Research Laboratory, School of Materials Science and Engineering, National Institute of Technology Calicut, India
² Department of Mechanical Engineering, National Institute of Technology Calicut, India
E-mail: hanas@nitc.ac.in

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Abstract

The biodegradability and mechanical properties of magnesium make it suitable for temporary implant applications. However, its degradation rate in the physiological environment needs to be controlled. The effect of grain refinement on the degradation rate in the physiological environment is investigated in this work. Samples with different grain size were developed by heat treatment and friction stir processing (FSP) techniques. Potentiodynamic polarization test in NaCl solution and immersion test in supersonic body fluid (SSBF) were conducted to evaluate the degradation resistance of the samples. The effect of grain refinement on bio-mineralization was also studied by analysing the surface morphology and composition of the immersion tested samples using SEM and EDAX. It is noted that the grain refinement improves the degradation resistance as well as bio-mineralization. The enhancement in the bio-mineralization resulted in the development of a calcium phosphate layer on the surface during the immersion test, which in turn reduced the degradation rate further. Hence, grain refinement can be used as an effective metallurgical modification technique to tailor the degradation rate of Mg–Ca alloys in the physiological environment.

1. Introduction

Orthopaedic implants are used to replace or support a damaged bone tissue or joint. These internal fixators are mostly in the form of plates, screws, nails, or wires generally made of stainless steel and titanium alloys [1–4]. In the case of temporary metallic implants, the use of these conventional materials necessitates a second surgery to remove the implant after recovery. Also, the high modulus values of these alloys compared to human bone results in stress shielding effect. This will lead to loss of bone density and it can delay the healing of tissues. Both of the above issues can be addressed using biodegradable materials like magnesium or its alloys [5–8]. However, pure magnesium does not have the required strength and hence magnesium alloys are preferred [9]. Though there are various magnesium alloys available in the market none of them is developed for implant applications in the physiological environment. The biocompatibility of the alloying elements needs to be considered while designing the alloys. The allowable per day limit for calcium (Ca) in the human body is more than 1000 mg/day [10]. This makes Ca one among the most suitable alloying elements for implant applications. However, it is reported that the corrosion rate of Mg–Ca alloys will increase rapidly with an increase in the Ca content [11, 12]. It is in this context, where the low Ca-containing magnesium alloys and their degradation control in the physiological environment become significant.

The degradation rate can be controlled by various techniques such as metallurgical modifications, surface modifications and protective coatings [13–15]. While the metallurgical and surface modifications can offer better surface stability, the coatings have the advantage of providing a wide range of composition and morphology. Song et al.[13] improved the degradation resistance of Mg–Zn implant with calcium-phosphate coating by electrodeposition. But the hydroxyapatite coated by electrodeposition was fragile, easily breakable and less stable. Li et al.[14] modified the Mg–Ca alloy surface by severe mechanical attrition treatment (SMAT) and achieved grain refinement. However, the degradation rate increased due to the strain-induced crystalline...
defects. Seong and Kim [15] achieved a reduction in the degradation of Mg–Ca alloy by high-speed differential rolling. This improvement was attributed to the reduction in grain size and uniform dispersion of intermetallic phases. Hence it is important to identify the optimum grain size which can reduce the degradation rate in the physiological environment. Moreover, studies have also reported that a reduction in grain size can improve the bioactivity of Mg alloys [16]. Such improvement in bioactivity can lead to accelerated nucleation and in situ growth of calcium phosphate (CaP) on the implants. Such a layer not only makes the surface conducive for cell adhesion and proliferation but also controls the degradation by protecting the surface beneath it. In the present work, the effect of grain size on biodegradation and biomineralization of low Ca containing Mg alloy in the physiological environment is investigated. Samples with different grain size were developed using heat treatment and FSP techniques. The performance of the samples in the physiological environment was studied by conducting immersion test in SSBF. The composition and morphology of surfaces, weight change of the samples, biomineralization, and variation in pH and degradation rate during immersion test were analysed to evaluate the performance of the samples.

2. Materials and methods

2.1. Materials

Mg–Ca alloy of chemical composition 0.66% Ca and remaining Mg was supplied by M/S NEXTGEN steel and alloy, Mumbai, India. All the chemicals and reagents used were of laboratory-grade supplied by Fisher Scientific.

2.2. Sample preparation

The samples of required dimensions (25 × 25 × 5 mm³) were cut and polished using emery papers up to 2000 grade followed by diamond paste. The polished samples were washed in deionized water and cleaned using acetone to remove any surface contaminations. These samples were annealed at a temperature of 380 °C for 1 h before further processing.

The required numbers of samples were then subjected to the heat treatment process for obtaining different grain size. All the samples were heated to 515 °C for 1 h in a muffle furnace (Vindish Instruments—EIE 1502, India). Predetermined numbers of samples were subjected to either furnace cooling, air cooling or water quenching in order to obtain the three different grain sizes. As the heat treatment method cannot yield ultrafine grain size another set of samples were also subjected to friction stir processing (FSP) on a milling machine (Batliboi—FA3V, India) using an H13 tool with a tapered pin. Figure 1 shows the schematic diagram of the FSP tool—workpiece assembly and photos of samples prepared for various process parameters. The FSP tool had a shoulder dimension of 20 mm and a tapered pin of length 3 mm with a diameter varying from 3 mm at the broader side to 1 mm at the tip. The process parameters like rotational speed, translational speed and depth of penetration of tools were varied as in table 1. A uniformly deformed defect-free surface corresponding to trial 3, as shown in figure 1(b)) was attained at a rotational speed of 2000 rpm with a depth of penetration of 0.2 mm and a translational speed of 14 mm min⁻¹.

2.3. Surface characterization

The microstructure of the processed samples were observed after proper polishing and etching with a picric acid solution using metallurgical microscope (Olympus BX51, Japan) and field emission scanning electron microscope (FESEM- Hitachi SU6600, Japan). The water contact angle of the sample surfaces were measured using Digidrop-MCAT goniometer (GBX, France). Vickers Hardness Number (Micromach MMV-D, India) of the unprocessed and grain refined samples were evaluated with a load of 100 g for 10 s. The elemental compositions of the sample surfaces after the immersion test were identified using SEM fitted with EDAX (OXFORD XMX N, UK).

2.4. Degradation Behaviour

2.4.1. Potentiodynamic polarization (PDP) test

PDP apparatus (CH instrument electroanalyser CH1608E, USA) consists of a three-electrode cell of which Mg–Ca alloy was used as the working electrode, platinum as the counter electrode and calomel as the reference electrode. 8.035 g l⁻¹ sodium chloride (NaCl) solution in deionized water was used as the electrolyte. Polarization curves were obtained by running the scan at a rate of 0.01 V s⁻¹. Corrosion current density is obtained from Tafel extrapolation and from corrosion current density, the corrosion rate (mm/year) can be measured by using equation (1),
Corrosion Rate \( = \frac{0.00327 \times EW \times I}{D} \)  

Where EW is the equivalent weight in g, D is the density of the alloy in g cm\(^{-3}\), and I is corrosion current density in \( \mu \text{A cm}^{-2} \). Among the heat treated samples, the one with the highest corrosion resistance was chosen as the optimum sample for further studies.

### 2.4.2. Immersion test

FSPed sample, the optimum sample from heat treatment and the unprocessed sample were selected for the immersion test. During the test, the temperature was maintained at 37°C with the help of a constant temperature water bath. Specimens were of dimension 10 x 10 x 3 mm\(^3\) and finely polished. Supersaturated simulated body fluid (SSBF) having ionic concentration five times that of human blood plasma was used as the medium for immersion test. The SSBF was prepared as reported by Kokubo and Takadama [17]. The initial weight and dimensions of the samples were noted before immersion. The samples were immersed for predetermined durations of 6 h, 12 h, 24 h, 48 h, and 72 h. The pH variation of the medium, weight of the samples after immersion and the weight after removing the degradation products was also recorded. To remove the degradation product, the samples were washed with boiling chromic acid. The degradation rate of samples in mm/year was calculated using equation (2),

\[
\text{Degradation Rate} = \frac{8.76 \times 10^4 \times W}{A \times T \times D}
\]
Where $W$ is the weight loss of specimen in g, $A$ is the area of the specimen in cm$^2$, $T$ is immersion time in h. The change of weight after washing the specimen with chromic acid gave the measure of deposition on those samples during the particular duration of immersion. The elemental composition of the biominerals present in the deposit was found using EDAX and their distribution was studied using SEM.

### 3. Results and discussions

#### 3.1. Microstructure studies

The microstructures of the processed samples are shown in figure 2. Among the heat treated samples, the water cooled, air cooled and furnace cooled samples showed an average grain size of 164.877, 361.958 and 607.2 μm respectively. It is thus evident that an increase in the cooling rate led to a reduction in the grain size. The growth of grains gets arrested during rapid water quenching process whereas, in furnace cooling, grain growth occurs as the heat dissipation is slower and the grains are at a higher temperature for longer durations. The cooling rate of the air cooled sample was between that of the furnace cooled and water quenched and hence exhibited a grain size value between the two. Among all the samples, FSPed sample showed the smallest average grain size of 13 μm due to dynamic recrystallization [9, 18]. The SEM image of the samples shown in figure 3 also confirms the grain refinement during heat treatment as well as FSP without any secondary phase formation.

#### 3.2. Wettability measurements

The effects of grain refinement on the wettability of the samples as given by contact angle measurements are shown in figure 4. The highest contact angle was recorded by the unprocessed sample and the lowest by FSPed sample. The samples with smaller grain sizes have good wettability. This increase in wettability is due to the increase in surface energy. As the grain size decreases the volume of grain boundary increases which increases the surface energy of the samples [19].

#### 3.3. Hardness test

The Vickers hardness values recorded for the samples were 35.37, 37.1, 37.53, 41.87 and 50.1 respectively for unprocessed, furnace cooled, air cooled, water cooled and FSPed samples. It is seen that the hardness of the samples increased with the reduction in the grain size. This increase in hardness can be explained based on the volume of grain boundaries and dislocation density. For the heat treated samples, there was not much difference in dislocation density but the volume of grain boundaries increased with the reduction in the grain size. This reduction in the grain size can lead to the strengthening of the alloy as per the Hall-Petch relation [20, 21]. In the case of FSPed sample, the increase in dislocation density leading to work-hardening also added to the strengthening of the sample along with the grain boundary effects. Hence FSPed sample recorded the highest hardness value.
3.4. PDP test
The potentiodynamic polarization curves of the samples are shown in figure 5. The corrosion current density, corrosion potential, and corrosion rate are tabulated in table 2. It is seen that the reduction in grain size helps in reducing the corrosion rate in the NaCl solution. The FSPed samples recorded the least corrosion rate compared to other samples. Among the heat-treated samples, the water-cooled sample showed the lowest corrosion rate. This reduction in corrosion rate is due to the reduction in the intensity of galvanic potential between grain and grain boundaries [22, 23]. Also as explained in section 3.2 the increase in surface energy of the sample, accelerate the formation of protective Mg(OH)₂ layer on the surface in the aqueous medium and thus reduce the corrosion rate.

3.5. Immersion test
3.5.1. In vitro biomineralization
Figure 6 shows the surface morphology and elemental composition of the sample surface before and after immersion in SSBF. The EDAX before immersion showed similar peaks for unprocessed, water cooled and FSPed samples (only Mg and O). After immersion, it is seen that all the samples had products of
biomineralization deposited on the surface. EDAX revealed that these depositions have Mg, O, Ca, P, Cl, and Na containing phases. The Mg is primarily from the sample and oxygen is primarily from the Mg(OH)$_2$ layer formed during the immersion. The Ca and P confirm the formation of CaP on the surface and the Cl$^{-}$ ions indicates the formation of MgCl$_2$. The amount of deposition was found to be least on the unprocessed samples. Among the grain refined samples, the FSPed sample had more deposition on the surface. SEM images also showed surface cracks on the Mg(OH)$_2$ layer formed after immersion. FSPed samples showed fewer amounts of

**Figure 5.** Potentiodynamic polarization curves.

**Figure 6.** SEM and EDAX of surface depositions on unprocessed, water cooled and FSPed samples during different immersion times.

**Table 2.** Corrosion Potential, corrosion current density and corrosion rate of all samples.

| Sample       | Corrosion potential (V) | Corrosion current density ($\mu$A cm$^{-2}$) | Corrosion rate (mm year$^{-1}$) |
|--------------|-------------------------|---------------------------------------------|--------------------------------|
| Unprocessed  | $-1.388$                | $5.794$                                     | $0.131$                        |
| Furnace cooled| $-1.516$                | $4.789$                                     | $0.108$                        |
| Air cooled   | $-1.469$                | $4.427$                                     | $0.1002$                       |
| Water cooled | $-1.512$                | $3.172$                                     | $0.072$                        |
| FSP          | $-1.509$                | $2.769$                                     | $0.063$                        |
cracks on the surface when compared to unprocessed and water cooled samples. As the immersion duration increased from 6 h to 72 h the extent of surface crack also increased which resulted in the partial removal of depositions from the surface.

The weight gain of the samples during the immersion test is shown in figure 7. This is due to the deposition of biomineralization products on the sample surfaces. The deposition was found to be highest in FSPed sample, followed by the water cooled sample. The increased surface energy of grain refined samples makes the specimen more hydrophilic and improves its wettability. Moreover, the fine-grained samples are more bioactive and promote biomineralization. Similar results were reported on other Mg alloys by Sunil et al [24] and Song et al [25]. They attributed the improved biomineralization to the increased amount of grain boundaries. The grain boundaries being high energy regions acted as sites for nucleation and thereby promoted CaP layer formation. It was observed that the CaP layer formed on the fine grained sample was more uniform compared to other samples. This is achieved due to the fine grain morphology of the FSPed samples [25]. As a result, the adherence of minerals and corroded products on FSPed samples are more compared to unprocessed samples. For all the samples, the deposition rate is higher in the initial period of immersion due to the rapid degradation reactions due to the high reactivity of Mg.

### 3.5.2. In vitro biodegradation

Figure 8 shows the surface morphology of immersed samples after the removal of surface depositions. After 6 h duration, the furnace cooled and water cooled samples showed more pits; meanwhile, the pits formed on the FSPed sample were shallow and less in number. After 72 h of immersion, the unprocessed samples showed severe pitting corrosion. The extent of pitting on the water cooled samples was less compared to the unprocessed samples. The FSPed samples showed least pitting corrosion characteristics after 72 h of immersion. This difference in pitting corrosion can be explained based on the in situ protection achieved by the samples during the immersion test [13, 16]. As explained in the section 3.5.1 the formation of water-soluble MgCl₂ leads to the pitting corrosion in all the samples. However, the extent of degradation due to pitting corrosion was minimal in the case of FSPed samples due to the enhanced biomineralization. The CaP layer formed on this surface protected the surface underneath from degradation. Among the heat-treated samples, the biomineralization was higher for water cooled samples and hence showed less number of pits compared to unprocessed samples.

### 3.5.3. pH Variation

The pH variation of SSBF during immersion test is shown in figure 9. All the samples recorded an increase in the pH during the immersion test. The pH of the medium with FSPed, water cooled and unprocessed samples increased from 7.22 to 7.827, 8.003 and 8.53 respectively after 72 h of immersion. When magnesium degrades, OH⁻ ions will be released to the medium, thereby increasing the pH value [26]. This increase in local pH during longer immersion time is due to the degradation of Mg(OH)₂ as reported elsewhere [16, 27]. When the degradation rate is higher, the release of these ions and correspondingly rise in pH will also be higher. The pH variation of the FSPed sample was least compared to other samples due to the low degradation rate as
discussed before in section 3.5.2. The unprocessed sample showed the maximum variation in pH as there was no protection on the surface.

3.5.4. In vitro degradation rate

Figure 10 shows the degradation rate of unprocessed and modified Mg-Ca alloy in SSBF during the immersion test. The degradation rate of all the samples reduced with increase in immersion duration. This rate of reduction was found to be higher during the initial stages of immersion which is due to the rapid formation of Mg(OH)$_2$ layer. This Mg(OH)$_2$ layer provided a stable surface for nucleation and growth of CaP through biomineralization. Generally, high energy grain boundary areas are the preferred corrosion sites and hence the FSPed samples are expected to degrade at a rapid rate than unprocessed samples. However, these preferred corrosion sites led to the rapid formation of the hydroxide layer resulting in a lower degradation rate than any other samples [16]. Also, as the immersion time increased, the enhanced bioactivity of the fine grained sample promoted biomineralization on the surface to form a CaP layer, which helped in further reduction of the degradation rate. Hence the FSPed sample showed the lowest degradation rate compared to other samples during the immersion test. The unprocessed sample surface was not conducive for development of CaP layer due to the development of cracks in the Mg(OH)$_2$ layer and pitting corrosion as discussed in the section 3.5.1. Thus the degradation rate of the unprocessed sample remained the highest during the immersion test.
4. Conclusion

Mg-Ca alloy with different grain sizes was developed through heat treatment and FSP. FSP produced an average grain size of 13.56 μm with improved wettability and hardness. The FSPed samples have a low degradation rate and better biomineralization capability compared to other samples. A reduction of 12.8% in degradation rate was obtained during the initial period of immersion. Hence microstructure modification by grain refinement can be used to tailor the biodegradation rate of Mg–Ca alloys.

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ORCID iDs

Sheeber A Rahim @ https://orcid.org/0000-0002-0739-7966
T Hanas @ https://orcid.org/0000-0002-9929-2490

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Figure 10. Degradation rate in SSBF during immersion test.
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