Tailoring biomineralization and biodegradation of Mg–Ca alloy by acetic acid pickling

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Abstract
Magnesium and its alloys are suitable candidates for developing biodegradable metallic implants. However, the rapid degradation of these alloys in the physiological environment is a major limitation for such applications. In this work, Mg–Ca alloy was chemically treated with acetic acid and its effects on degradation behaviour were studied using simulated body fluid (SBF). The surface morphology and composition of the acid pickled samples were investigated using a scanning electron microscope (SEM) and infrared spectroscopy (IR). The degradation rate was analysed by conducting potentiodynamic polarization (PDP) and immersion tests. The results show that optimum acetic acid treatment improved the corrosion resistance by acid etching and formation of magnesium acetate layer. The treated samples also exhibited enhanced biomineralization and developed calcium phosphate layer on the surfaces during immersion tests. It is proposed that acetic acid pickling can be used as a reliable technique for surface modification as well as for pre-treatment of magnesium alloys to make them suitable for degradable metallic implant applications.

1. Introduction

The superior mechanical properties of metallic materials over ceramics and polymers make them suitable candidates for load-bearing orthopaedic implants such as bone rods, pins, plates, and screws [1–3]. Conventionally, these metallic implants are made from stainless steel and Ti-based alloys. When used for temporary applications these alloys necessitates a second surgery for removal of the implant after the tissue is healed [4, 5]. This can lead to patient morbidity and increase in cost for healthcare. Furthermore, the global population together with elderly people having a high risk for bone–tissue failure is rapidly increasing. Hence, it is very essential to develop biodegradable metallic implants that can improve the quality of life (QOL). The advances in materials science and technology have created the possibility of developing degradable implants from metallic materials in the near future. Statistical analysis of published scientific journals over the last 15 years as shown in figure 1 highlight that biodegradable metallic materials are among the forefront research topics of biomaterials.

Magnesium (Mg) and their alloys are possible candidates for temporary orthopaedic implant applications as they are biodegradable, biocompatible and possess mechanical properties close to that of human bone [6, 7]. However, the high reactivity of Mg leads to rapid degradation, hydrogen gas evolution, and poor mechanical integrity in the physiological environment [7, 8]. Recently, many attempts were made to improve the degradation resistance of Mg-based alloys, mainly by metallurgical and/or surface modifications. The metallurgical modification mainly includes alloying [9, 10] and grain refinement by various plastic deformation processes [11, 12]. The addition of alloying elements can improve the degradation behaviour of Mg to a greater extent. However, biosafety being an important factor, choosing the Mg-alloys based on toxic-free constituent elements is very critical. The alloys containing aluminium (neurotoxicant) [13, 14] and rare earth elements (forms colloid in the blood) [13, 15] can trigger serious issues for the neighbouring tissues. Li et al reported the acceptability of binary Mg–Ca alloys for biodegradable implants having suitable degradation rate due to
enhanced mineral deposition [16]. Though Ca was effective to inhibit degradation rate, its higher concentration can cause detrimental effects by accelerating the degradation [17]. The various surface modifications attempted include conversion coatings [18], deposition of thin films by various coating techniques [19–22] and mechanical processing of the surfaces [23–25]. The mechanical methods have a demerit of causing surface impurities that accelerate degradation by creating galvanic couples in the sample surface [26, 27]. Though there are several coating techniques, conversion coating by acid pickling is a simpler and cheaper method to achieve controlled degradation rate for biomedical applications. The thin films developed through specific reactions between the sample surface and acidic medium during pickling will have better adhesion. Furthermore, the surface impurities during the processing of metallic alloys that causes micro-galvanic corrosion can be reduced due to the acid etching. Nwaogu et al reported that the treatment with various organic and inorganic acids controlled the degradation rate of AZ31 alloy due to reduced micro-galvanic cell formation and deposition of protective layers on the surface [28, 29]. Among the organic acids, the best degradation rate was obtained with acetic acid pickling [28, 30]. However, no relevant studies focused on the effect of acid treatment on biodegradation and biomineralization of Mg alloys have been reported so far. Unlike the conventional systems, the physiological systems can promote the formation of in situ biomineralization coatings on the samples which can also help in controlling the degradation rate.

In this study, the acetic acid pickled samples with maximum corrosion resistance were chosen for the immersion test in simulated body fluid (SBF) to evaluate its potential for biodegradable orthopaedic implant applications. The effect of acid treatment on surface morphology, biomineralization and degradation rate of Mg–Ca alloys were compared with that of the untreated samples. The study proposes an optimum acetic acid treatment process which can be used to achieve better biomineralization and improved degradation resistance.

2. Experimental procedures

2.1. Sample preparation
Mg–Ca alloy with low Ca content (<0.6%) was supplied by M/S Nextgen Steels and Alloys, Mumbai, India. Other chemicals and reagents of laboratory grade were supplied by Fisher scientific. The samples were cut and the surfaces were subjected to belt-grinding to the required size and dimension. The samples are then annealed at 340 °C for 1 h and finely polished to 1000 grade using silicon carbide papers. The polished samples were then washed in ethanol and dried in air. These samples were treated in acetic acid of 1 molar concentration for different durations. The samples were then coded as M-A, A-A30, A-A60, A-A90, A-A120, and A-A180; where M-A stands for annealed bare sample, A-A for the acid-treated sample and the numbers corresponds to their treatment time in seconds (s).

2.2. Surface morphology and characterisation
The sample surface morphology and compositions were characterized using a scanning electron microscope (FESEM, Hitachi SU6600, Japan) and Energy dispersive spectroscopy (EDS). The functional groups formed on the surface during processing were studied by Fourier transform infrared (FTIR Frontier, PerkinElmer, USA).
analysing the extent of biomineralization as weight gain noted. The weights of the samples without removing surface depositions were recorded after immersion for as reported by Kokubo. The material removal in \( \mu \text{m} \) during acid pickling as a result of etching was also calculated for optimizing the treatment time as per the following equation (2):

\[
\text{Material removal} = \frac{w_a \times 10^6}{D \times A_o}
\]

where \( w_a \) is the change in weight (g) before and after acid pickling corresponding to various treatment time and \( A_o \) is the initial surface area in \( \text{mm}^2 \).

2.3.2. Immersion test

The performance of the acid pickled sample with the highest corrosion resistance as seen from PDP results was compared with that of the annealed samples by conducting immersion test in SBF. The in vitro test for aforesaid samples was conducted for different immersion durations of 3, 7, 14 and 28 days in SBF. The SBF was prepared as per the following equation (3):

\[
\text{Degradation rate} = \frac{8.76 \times 10^4 \times W}{A \times T \times D}
\]

where \( W \) is the total weight loss (g) for a duration of time \( T \) (h) and \( A \) (cm\(^2\)) is the surface area before immersion in SBF.

3. Results

3.1. Surface morphology and characterisation

The SEM images of the samples before and after acetic acid pickling is shown in figure 2. The surface of Mg–Ca alloys after pickling in acetic acid had a bright greyish appearance. The rough grinding marks as seen in the case of annealed samples (figure 2 c) were etched during the acid treatment process as evident from the image of treated samples. The SEM image for the treated sample at lower magnification confirms the surface etching occurred during acid pickling. This etching action can clean the impurities from the surface [28]. But the removal of impurities depends on the immersion time as complete cleaning of the surface does not occur at shorter immersion time. The optimum immersion time can be obtained using the PDP test results as the samples without surface contamination is expected to exhibit the lowest corrosion rate.

The treated sample also shows a thin uniform deposition over the surface and was analyzed by FTIR as shown in figure 3. The bare magnesium alloy had no significant peaks for the range 500 to 4000 \text{ cm}^{-1}, indicating that no functional groups were present on the surface [20, 33, 34]. The FTIR spectra of acid pickled sample exhibits a peak at 2921 \text{ cm}^{-1} which matches with C–H stretching and another peak at 1405 \text{ cm}^{-1} attributed to C–H bending. The peak at 1024 \text{ cm}^{-1} indicates C–O stretching due to the presence of carboxylic acid groups in...
the sample. The peak at 1559 cm$^{-1}$ is assigned to the asymmetric stretching mode of –COO functional groups present in magnesium acetate [35]. The presence of aforesaid peaks confirms the formation of protective magnesium acetate layer. Furthermore, the broad band near 3280 cm$^{-1}$ indicates the vibration of hydroxide groups (OH) and the peaks at 860, 515 cm$^{-1}$ are assigned to the formation of oxides (MgO) [36, 37]. The hydroxide and oxide layers can provide additional protection on the substrate surface.

3.2. Potentiodynamic polarization test
The polarization curves of the samples obtained by conducting PDP tests are shown in figure 4. The curve shows an anodic shift in corrosion potential for samples A-A120 and A-A180. This improvement in the corrosion resistance of the samples treated with acetic acid for longer duration is mainly due to the formation of magnesium acetate layer. As the immersion time increases more reaction happens and it leads to the formation of a thin and uniform magnesium acetate layer on the surface which can enhance the corrosion resistance. However, for shorter immersion time, there was no significant improvement in the corrosion rate (figure 5). It may also be noted that the ICP analysis of the samples showed the presence of Fe, Al, Mn, Si, and Zn as impurities. As reported by many researchers, acid treatment can remove such impurities present on the surface of the samples and avoid the formation of micro-galvanic cells [28–30].

The surface morphology of the corroded area of acetic acid treated samples during PDP test is shown in figure 6. The morphology shows the presence of corrosion deposits on annealed samples in comparison with acid treated samples. The bare Mg-based alloys exposed to aqueous solution form magnesium hydroxide which reacts with chloride ions to form highly soluble magnesium chloride (MgCl$_2$) [7, 38, 39]. This MgCl$_2$ deposit accelerates the pitting corrosion of untreated samples. However, the acid treated samples confirm the presence of chemical conversion coatings even after the exposure to a corrosive environment.
3.3. Material removal

The weight loss exhibited by the samples during acid pickling is expressed in terms of material removal as shown in figure 7. The material removal is due to the acid etching phenomenon from the surface. As expected, the...
material removal increased with treatment time. Hence, to avoid a higher weight loss due to the acid treatment, A-A120 samples were preferred over A-A180 for further immersion test in SBF. It may be noted that there is no significant difference in the degradation rate of A-A120 and A-A180 samples as explained in section 3.2.

3.4. Immersion test

3.4.1. In vitro biomineralization
The SEM and EDS of the phases present in M-A and A-A (A-A120) samples before and after immersion in SBF are shown in figure 8. The peaks corresponding to Mg are from the alloy and presence of O and C confirms the formation of hydroxide and acetate phases. It was also noted that Ca was not detected by EDS from both the samples before immersion. This is due to the low volume fraction of Ca in the samples. However, the EDS results after immersion showed the presence of Ca, P and more O in both the samples which indicates the formation of calcium phosphate (CaP) layer due to biomineralization. Even though both samples had a similar composition, the relative quantities were quite different. The A-A samples showed more amount of Ca and P than M-A samples. The presence of O also suggests the formation of protective Mg(OH)₂ layers which reduces the degradation rate. But, they are highly soluble in aqueous media like SBF. The Cl ions present in the SBF leads to the formation of MgCl₂ from Mg(OH)₂. MgCl₂ is soluble in aqueous media and results in pitting corrosion.
Also, the relatively high intensity of Cl for M-A samples indicates the severity of pitting corrosion and the SEM images of their surface show more cracks after 28 days of immersion. Conversely, A-A samples showed fewer cracks and more biomineralization indicating better resistance to pitting corrosion.

Figure 9 shows the weight change due to deposition of biomineralization and corrosion products. During the initial days, both samples exhibited weight loss. The M-A samples exhibited weight loss throughout the immersion test due to degradation resulting in the removal of deposits and crack formation (figure 8). The A-A samples showed weight gain after 28 days of immersion indicating deposition of some phases as a result of better biomineralization. The improvement in biomineralization of A-A samples is attributed to the clean and stable surface obtained through acid treatment [40, 41]. The stable surface with magnesium acetate layer acted as a scaffold for nucleation and growth of CaP through biomineralization.

3.4.2. In vitro degradation

The SEM images of the cleaned sample surfaces immersed in SBF for different durations are shown in figure 10. M-A samples showed deeper pits from the third day of immersion. The formation of these pits had triggered pitting corrosion as the new surface gets exposed to Cl ions. But, the extent of pitting was minimal for A-A samples during the initial days attributed to the formation of magnesium acetate deposition that acted as a protective layer. However, magnesium acetate layer is soluble in aqueous solution and pitting was observed for A-A samples after 3 days of immersion. Both samples revealed layer by layer removal of materials during longer immersion time.
The degradation rate of the samples during the immersion test in SBF is graphically illustrated in figure 11. As expected, the A-A samples exhibited the least degradation rate at all intervals of time. In fact, the degradation rate is lesser than the values in figure 11, as the chromic acid treatment, will remove the magnesium acetate layer.
too. Initially, there was rapid degradation for both samples due to the formation of cracks from the surface. The degradation rate decreased with increasing time, indicating the formation of the CaP layer due to biomineralization.

4. Discussion

The rapid degradation of Mg-based alloys due to the Cl\textsuperscript{−} attack is associated with hydrogen gas evolution, changes in the local pH and severe pitting corrosion [7, 42]. The surface modification had a major influence on controlling the degradation mechanism of Mg–Ca alloys. Figure 12 schematically represents the degradation mechanism for samples with and without acid treatment. In the case of M-A samples having no protective layers, there is pitting from the initial days of immersion due to the Cl\textsuperscript{−} attack. The pitting becomes severe during the later stages of immersion. Conversely, the magnesium acetate layer for A-A samples provided immediate protection from the initial days of immersion. Moreover, the layer acted as a scaffold for nucleation and growth of CaP layer and thus promoted biomineralization. But with the increase in immersion time, the acetate layer and CaP deposits were deficient to completely mask the sample surface from Cl\textsuperscript{−} attack. Hence moderate pitting was observed during the later stages of immersion. This pitting can be attributed to the low Pilling-Bedworth (PB) ratio (∼0.8) of magnesium alloys [43]. However, the acetic acid treatment can be further combined with suitable deposition coating techniques to bring a synergetic effect for tailoring biodegradation and enhancing biomineralization of Mg–Ca alloys.

5. Conclusion

The acetic acid treatment for Mg–Ca alloy resulted in reduced corrosion rate due to the acid etching and formation of acetate layer. During the immersion test, the magnesium acetate layer on the surface also reduced pitting corrosion in the initial stages of immersion test and made the sample surface stable for nucleation and growth of biomineralization products. This enhancement in biomineralization along with degradation resistance due to acid pickling can be combined with suitable surface deposition coatings for tailoring the degradation rate of Mg–Ca alloys for biodegradable orthopaedic implant applications.

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