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Friction stir processing of magnesium–nanohydroxyapatite composites with controlled *in vitro* degradation behavior

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ABSTRACT

Nano-hydroxyapatite (nHA) reinforced magnesium composite (Mg–nHA) was fabricated by friction stir processing (FSP). The effect of smaller grain size and the presence of nHA particles on controlling the degradation of magnesium were investigated. Grain refinement from 1500 μm to ≈3.5 μm was observed after FSP. *In vitro* bioactivity studies by immersing the samples in supersaturated simulated body fluid (SBF 5×) indicate that the increased hydrophilicity and pronounced biomineralization are due to grain refinement and the presence of nHA in the composite respectively. Electrochemical test to assess the corrosion behavior also clearly showed the improved corrosion resistance due to grain refinement and enhanced biomineralization. Using MTT colorimetric assay, cytotoxicity study of the samples with rat skeletal muscle (L6) cells indicate marginal increase in cell viability of the FSP-Mg–nHA sample. The composite also showed good cell adhesion.

1. Introduction

Metallic biomaterials are mostly preferred for load bearing applications compared to ceramics and polymers. Especially for temporary tissue applications, magnesium based materials are the promising choice due to its degradability in physiological environment and load bearing capacity. This avoids the subsequent operation to remove the implant after healing of the fractured bone [1–4]. The mechanical properties of magnesium are also closer to natural bone which minimizes stress shielding effect that is generally associated with other metallic systems. But magnesium corrodes quickly in physiological environment and produces magnesium hydroxide by evolving hydrogen during the process of degradation [5,6]. If the rate of degradation is low, the produced hydrogen gas is absorbed by the tissue as it forms. If the degradation is too rapid, the gas may form a sub-facial pocket at the implant and tissue interface. However, it has been reported that the hydrogen gas evolved during the degradation of magnesium completely disappears after 6 weeks and generally does not cause complications [2,7]. But the material–tissue interactions that depend on the cell activities are affected due to the rapid degradation [8]. So, controlling the rapid degradation is the critical issue in developing magnesium based biodegradable implants. The degradation rate of magnesium can be altered by developing new alloys and composites, surface coatings and modifying the microstructure [9–12]. Hydroxyapatite (HA), a calcium phosphate mineral phase, has attracted a great interest for the past two decades due to its excellent biocompatibility, bioactivity and osseointegration [13]. There are few reports on the fabrication of magnesium–HA composites from powder metallurgy route [14,15]. Also, considerable work has been done on coating the surface of different magnesium alloys with HA to improve the corrosion resistance [10]. However, as the degradation initiates from the surface of the magnesium, introducing HA into the surface can give better protection since it makes the substrate itself more bioactive.

In the present paper, we demonstrate the use of friction stir processing (FSP), a solid-state processing technique developed from friction stir welding (FSW) [16] to disperse nano-hydroxyapatite (nHA) particles into pure magnesium to fabricate fine grained Mg–nHA composite. In FSP, a cylindrical rotating tool consisting of a small pin is inserted into the material surface and moved to cause dynamic recrystallization due to intense plastic deformation resulting in significant grain refinement [17]. The stirring action of the FSP tool can be used to fabricate metal matrix composites by incorporating secondary phase particles during the process. Mishra et al. [18] explained the composite fabrication using FSP in preparing 5083Al-based SiC reinforced surface composite and subsequently, significant work has been reported on the formation of different metal matrix composites (MMCs). Table 1 lists the brief development in magnesium based composites using FSP. As evident from the table, the fabrication of magnesium based composites by FSP are limited and in particular, studies targeted for biomedical applications are lacking. In the present work, Mg–nHA composites were prepared using the FSP process. The effects of the fine grain structure and

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Table 1
List of work carried out to develop magnesium based composites by friction stir processing in chronological order.

S. no.	Composite	Study/observations	Reference
1	AZ91 Mg alloy/SiC powder	Achieved fine grain structure and improved wear properties	Lee et al. [19]
2	AZ61 Mg alloy/nano-SiO_2	Grain refinement, high hardness, high strain rate and superplasticity	Lee et al. [20]
3	Multi walled carbon nanotubes (MWCNTs)/AZ31 Mg alloy	Grain refinement and improved hardness	Morisada et al. [21]
4	Al-rich thixoformed AZ91D Mg alloy	Grain refinement and improved corrosion resistance	Chen et al. [22]
5	AZ91 Mg alloy/SiC powder	Effect of processing parameters on microstructure.	Asadi et al. [23]
6	AZ31 Mg alloy/nano-Al_2O_3	Effect of rotational speed and probe profile on microstructure and hardness	Azizieh et al. [24]
7	AZ91 Mg alloy/SiC and Al_2O_3 powders	Microstructural observations Hardness measurements	Asadi et al. [25]
8	i) AZ31B Mg alloy/carbon fibers ii) AZ91D Mg alloy/carbon fibers	Effect of the matrix characteristics, experimental parameters on the microstructural changes Fragmentation and distribution of the carbon fibers, recrystallization, solutionizing and precipitation	Martens et al. [26]

distributed nHA particles resulting from FSP on biomineralization, degradation and cytotoxicity to the rat skeletal muscle (L6) cells were investigated. The feasibility of the FSP technique to be adopted for biomedical applications is also discussed.

2. Materials and methods

2.1. Processing

Friction stir processing was performed on commercially available pure magnesium (M/s. Exclusive magnesium, Hyderabad, India) sheets of size 100 × 100 × 5 mm cut from the billet. Prior to the processing, the sheets were annealed at 340 ºC for 30 min followed by furnace cooling. Annealed pure magnesium was coded as Mg. The chemical composition of the pure magnesium as confirmed by inductively coupled plasma atomic emission spectroscopy (ICP-AES, Optima 5300DV, PerkinElmer, USA) is 0.003% Al, 0.001% Zn, 0.002% Fe, 0.008%Mn and the remaining being magnesium in atomic percentage. The tool used in the present study is made of hardened H-13 tool steel consisting a tapered pin with a diameter varying from 3 to 5 mm over 2.7 mm length. The shoulder diameter of the tool is 15 mm. Optimized parameters were obtained by processing with different combinations of load, tool speed and its traverse speed to attain defect free stir zone. The traverse speed of rotating tool along the traverse axis was 12 mm/min with a rotating speed of 1200 rpm. 5000 N load was applied during the process. The processed sample was coded as FSP Mg. In order to produce surface composite, a shallow groove of 1 mm width and 2 mm depth was machined on the surface of the Mg sheets using milling cutter and the groove was filled with nHA powder. The nHA powder used in the present study was synthesized by microwave irradiation method as reported by Rameshbabu et al. [27]. Then FSP was carried out with the pin and tool plunged into the groove. Same processing parameters used for FSP Mg sample were adopted to produce the composite and coded as FSP-Mg–nHA.

2.2. Characterization

Samples of size 20 × 10 × 5 mm were cut from the annealed sample and across the stir zone of FSPed samples for microstructural observations. They were then mechanically polished using emery papers up to 2000 grade and washed using 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). Then the polished samples were etched with a solution comprised of 5 ml acetic acid, 5 g picric acid, 10 ml water and 100 ml ethyl alcohol for 20 to 60 s. After polishing and before etching, the samples were cleaned ultrasonically in ethanol to remove any residues generated from polishing. The microstructural observations were carried out using optical microscope (Vertimet-CP, Chennai Metco, India), scanning electron microscope (SEM, FEI Quanta 200, Netherlands) operated at 30 kV. Energy dispersive X-ray (EDS) mapping was also carried out to confirm the distribution of calcium and phosphorous in the FSP-Mg–nHA sample. For TEM observations, samples were cut from the center of the processed zone and thin slices were cut from the samples using an automated slow speed saw. Then they were mechanically thinned to a thickness of 100 μm using fine graded emery papers. Then the thin disks were subjected to electro-polishing using a twin-jet electro-polishing facility using an electrolyte (mixture of 1% perchloric acid and 99% ethanol) until a hole was formed. The adjacent areas of these holes having a thickness less than 100 nm were examined by transmission electron microscope (TEM, Philips CM12, Holland) operated at 120 kV. Samples of weight 25 mg were cut from the stir zone as well as from the unprocessed regions and dissolved in 2% HNO_3 aqueous solution. The elemental composition of the solution was analyzed by ICP-AES method to assess the possible dissolution of iron from the FSP tool into the magnesium sheets during the process. For wettability studies, all the samples were mechanically polished using emery papers up to 2000 grade, cleaned with ethanol, dried and the contact angles were measured (Easy DROP, KRUSS, Germany) using distilled water as the solvent at five different locations under ambient conditions.

2.3. In vitro bioactivity

Specimens of size 10 × 10 × 5 mm were cut from the central part of the stirred zone and dried at 60 °C for 2 h after ultrasonic cleaning in ethanol. Super saturated concentrations (SBF 5×) have been used in the present study to accelerate the mineralization and to quickly assess the role of mineral phases on the degradation behavior of the samples. The samples were immersed in SBF 5× and kept in a constant water bath at a temperature of 37 °C for 72 h to study the bioactivity. The ion concentrations of SBF 5× are shown in Table 2 and compared with that of SBF as reported by Kokubo et al. [28]. The reagent grade chemicals (NaCl, $NaHCO_3$, KCl, K_2HPO_4, $MgCl_2 \cdot 6H_2O$, $CaCl_2$ and Na_2SO_4 (Merck, India.)) with an appropriate weight were dissolved in deionized water as per the recommended sequence. Then the solution was buffered at pH = 7.4 with tris-hydroxymethyl aminomethane (TRIS) and appropriate amount of 1 M HCl at 37 °C [28]. Each sample was immersed in 50 ml of SBF 5× solution (the ratio of the SBF volume to the sample apparent surface area is more than 1:10). After different

Table 2
Ion concentrations of the SBF 5×.

Ion	Ion concentrations (mM)		
	Blood plasma	SBF	SBF 5×
Na^+	142	142	710
K^+	5	5	25
Mg^{2+}	1.5	1.5	7.5
Ca^{2+}	2.5	2.5	12.5
Cl^-	103	147.8	739
HCO_3^-	27	4.2	21
HPO_4^{2-}	1.0	1.0	5.0
SO_4^{2-}	0.5	0.5	2.5
pH	7.2–7.4	7.4	7.4