



Study of dynamic degradation behaviour of porous magnesium under physiological environment of human cancellous bone

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ABSTRACT

This study analyse the effect of integrating of physiological environment of human cancellous bone as shown by different level of cyclic compressive on the degradation behaviour of porous magnesium under dynamic immersion for bone scaffold applications. The porous magnesium (30%, 41% and 55% of porosity) were immersed in simulated body fluid (SBF) with flowrate 0.0025 ml/min while having dynamic loading (1000 $\mu\epsilon$, 2000 $\mu\epsilon$ and 3500 $\mu\epsilon$) for 24, 48 and 72 h. The influenced integrating both boundaries have increased the relative weight loss and degradation rate as high as 61.56% and 93.67%, respectively as compared to dynamic immersion test only.

1. Introduction

Mechanical loading induced by the physiological activities is known to be the key factor in regulating bone tissue mechanism of adaptation and formation [1]. Both mechanical stimulation of fluid flow passing through cancellous bone structure and substrates strain generated by the external forces were identified as the physical stimulus that triggered the bone cells respond encouraging for bone modelling and remodelling [2,3]. Currently, biodegradable metals have been suggested for bone scaffold applications due to their mechanical properties better for load bearing applications [4–7]. The degradation behaviour of biodegradable metals are often depending on the measurement setup and environment type used to assess their potential for biomedical implant applications [8]. Bone scaffolds made of biodegradable metals are required to have porous structure which are allowing the tissues interlocking, cell migration and nutrients transport and osteo-integration with host tissues replaced [9]. Once the bone scaffolds implanted, it will prone to the mechanical stimuli from the host tissue. Therefore, the stimulus boundary of fluid flow and cyclic motion of bone strain are necessary to consider simultaneously in assessing the degradation of biodegradable metals for bone scaffolds application.

In contrast to the investigations rarely to Fe-based and newly introduced Zn-alloys, Mg and its alloys have most considered biodegradable metals for potential bone scaffold applications [8,10–16]. Mg have exciting characteristics of atypically lightweight metal with a density of 1.74 g/cm³ [17]. Mg hold interesting mechanical property closed to human bone, their Young's modulus is 41–45 GPa and cortical

bone is 3–23 GPa [18,19]. Mechanical property of Mg can be regulated and controllable precisely by constructing a porous structure to obtain closely match of cancellous bone Young's modulus of 0.01–3.0 GPa [20]. Mg is easily to be founded in human bone tissue since its function is essential for human metabolism [21]. It is stored in bone tissue almost half from the total physiological and fourth most considerable cation with 1 mol of Mg per 70 kg of adult body [10]. Due to their ionic naturally presence in human body with significant functional roles have facilitated the Mg-based scaffolds to serve as biocompatible, osteo-conductive and osteo-integration with surrounding tissues [22]. Moreover, from bioactivity point of view, magnesium has a stimulatory effect to bone growth due to the formation of bone-apatite like hydroxyapatite crystal which is favourable for bone strength [23–25].

Cancellous bone remodelling is continuously occur by the couple actions of osteoclasts and osteoblasts during resorption and formation process. The osteocytes are to be known as the orchestrator in bone remodelling process that initiate the cues and triggered for bone resorption and formation [26]. The mechanical loading from the cyclic motion of physiological activities is the regulator mechanism that excite the mechano sensitive of osteocytes [27]. The changes in mechanical stimuli which produced by the mechanical stresses is transduced by osteocytes into chemical signal that triggered the cellular response governing the bone modelling and remodelling [26,28]. During the physical routine activity such as walking, the pressure difference created have causes the bone marrow (home of progenitor cells) flow passing through the cancellous bone structure ranges from 0.0072–1.67 ml/min [29–33]. The changes in hydrostatic pressure is

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resulted by the deformation of bone matrix (strain) [34]. Bone structure adapts difference loading level (bone strain) that classified in three phases: lazy zone (bone resorption), remodelling equilibrium and critical damage caused bone to fracture. The lazy zone caused bone resorption due to disuse and its initiated 1000 micro-strain ($\mu\epsilon$) [35]. The remodelling equilibrium is generated from daily physical activity and exercise caused the loading level up to 3000 $\mu\epsilon$ [36,37]. The critical damage phase is generating the 3500 $\mu\epsilon$ of strains due to robust condition that caused bone fracture [38,39].

To date the biomechanical stimulus boundary of fluid flow and bone strain have been investigated by means of regulating the bone cells response in vitro cell culture systems and fluid structure interaction techniques [1,40–42]. Thus far, there is no study using both mechanical stimulus as boundary for degradation test using porous Mg in dynamic immersion test system. Therefore, the objective of this study is to investigate the influence of mechanical stimulus of fluid flow and bone strain on the degradation behaviour and mechanical integrity of the porous Mg using simulated body fluid (SBF) as fluid medium. Those boundaries of biomechanics of bone are believing to provide the more realistic condition once implanted as bone scaffolds.

2. Experimental procedure

2.1. Sample preparation

Porous Mg specimen material was from commercially pure Mg (99.9% purity) rod with a 25.4 mm diameter (Goodfellow Inc, Cambridge, UK) with the following composition (in wt.%): 0.028 Fe, 0.007 Al, < 0.002 Zn, 0.017 Mn, < 0.001 Ni, 0.005 Si, 0.002 Cu. The geometry of the specimen was specifically design with interconnectivity pore at varying porosity and surface area in rectangular prism shape of $5 \times 5 \times 3$ mm. The specimens were cut and drilled using an 800 μm diameter drill bit in a CNC machine (HAAS, USA). The interconnected holes (internal surfaces) of the specimen were cleaned using interdental brushes (Tepe, USA), 0.6 mm and 0.8 mm in size to remove any excess materials and chemicals. The external surface of the specimens was ground using an abrasive paper grit #800 and #1200. Then, the specimens were cleaned using ultrasonically in acetone for 15 min, rinsed with acetone, and dried in a vacuum chamber for 1 h before being subjected to immersion tests. The morphological indices of the specimens as describes in Table 1.

2.2. Dynamic immersion test

The porous Mg subjected to dynamic degradation test using simulated body fluid (SBF) as fluid medium. The 1 l of SBF solution was made by dissolving the reagents in sequence: 8.035 g NaCl, 0.355 g NaHCO₃, 0.225 g KCl, 0.231 g K₂HPO₄·3H₂O, K₂HPO₄·3H₂O, 0.311 g MgCl₂·6H₂O, 39 ml HCl 1.0 M, 0.292 g CaCl₂, 0.072 g Na₂SO₄, 6.118 g Tris-buffer. The 1–5 ml HCl 1.0 M was dropped into the solution to adjust the pH of SBF to 7.4 at 36.5 °C \pm 1 °C as reported by Kokubo [43]. The SBF passing through the specimen at 0.025 ml/min as a laminar flow using a peristaltic pump with flowrate capacity of

Table 1
The morphology of porous pure magnesium specimens [4].

Type	Porosity	Total surface area ^a	Exposed surface area ^b	Mass	Volume
A	30%	189.30 mm ²	135.34 mm ²	82.8 mg	52.87 mm ³
B	41%	209.81 mm ²	161.89 mm ²	70.3 mg	44.57 mm ³
C	55%	225.75 mm ²	181.83 mm ²	53.3 mg	33.83 mm ³

^a Total surface area is the whole area of the specimen's surface.

^b The exposed surface area was the area that exposed to the fluid. The different between the total surface area and exposed surface area is the two surfaces (top and bottom surface) that were directly contacted to the shaft and holder, respectively.

0.015–32 ml/min. A 250 ml tank of SBF immersed in water bath with temperature preserved at 37 °C \pm 1 °C using temperature controller (Shimaden, Japan) was connected to a pump using a 2 mm inner diameter silicone tube. Two pressure transducers (EMA, China) were used to measure the pressure difference before and after the specimen which the signals of the pressure was capture by a data acquisition (DAQ – National Instruments, USA).

2.3. Cyclic compression chamber

The test rigs as shown in Fig. 1 was designed to mimic the condition of cancellous bone subjected to the bone marrow movement and the perpetual motion of loading due to physiological activity. The acrylic chamber and stainless steel 316L (medical grade) shaft were fabricated using CNC machine (HAAS, USA). The chamber was specifically hold the porous Mg allowing the SBF pass through and subjected to cyclic loading via the shaft. This flowrate was selected represent the ideal state of bone marrow under normal condition [29]. Three different loading of 1000, 2000 and 3500 $\mu\epsilon$ were applied to the specimens. The cyclic loading was performed using strain-controlled test on the universal testing machine (The FastTrack 8874, Instron, Norwood, USA). The cyclic loading was controlled by a software (WaveMatrix™ Dynamic Materials Testing Software, Instron®, USA). All specimens were tested at a frequency of 2 Hz sine wave which related to the average normal walking condition range 1–3 Hz [36,44]. The degradation of secants modulus (E_N) at particular number of cycles was calculated based on the load-displacement data as shown in Fig. 2. The porous Mg were subjected under fluid flow integrate cyclic loading tests for 24, 48 and 72 h. After test, the specimen was removed from the chamber, gently rinsed with deionized water and dried under vacuum for 1 h. The total specimens subjected to the test was 81 specimen represents three repetitions from three types of porosity, immersion time and cyclic loading.

2.4. Materials characterization

The degradation products morphologies and chemical analysis on top of the surface specimens were observed and spotted using a field emission scanning electron microscopy (FESEM Supra 35-VP, Carl Zeiss, Germany) equipped with energy dispersive spectrometer (EDS), and X-ray diffractometer (XRD D5000, Siemens, Germany) using the Cu K α radiation at the step size of 0.02° with a scanning speed of 2°/min.

2.5. Mechanical testing

The tested specimens were scanned using micro computed tomography (μCT) (Skyscan 1172; Kontich, Belgium) at a resolution of 17 μm to identify the area contacted with compression jig for stress calculation. The 2-D planes data and the 3D model of the specimens were constructed using MIMICS software (Materialise, Belgium). The compression testing was performed using a universal testing machine (The FastTrack 8874, Instron, Norwood, USA). The mechanical properties of porous Mg specimens before and after immersion tests were evaluated under compression test, at a strain rate was of 0.005/s using a 25 kN load-cell. The compressive strength, yield strength and Young's modulus were determined by following the ASTM D1621 and ISO 844 standards.

2.6. Degradation rate determination

Degradation rate was determined by the method of weight loss measurement. This method has been deemed to be as gold standard in measuring corrosion of metal. In weight loss measurement, the mass and morphology of porous specimen is measured before and after dynamic immersion test for 24, 48 and 72 h. Once the test is completed, prior to measuring the mass of porous specimen, it is prerequisite to

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