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# A Comparison of Degradation Rate Bone Scaffold Morphology Between Computer Simulation and Experimental Approach

Akbar Teguh Prakoso<sup>a</sup>, Ardiyansyah Syahrom<sup>b,c</sup>, M. A. Sulong<sup>b,c</sup>, Amir Putra Md Saad<sup>b</sup>, Irsyadi Yani<sup>a</sup>, Jimmy D. Nasution<sup>a</sup>, Hasan Basri<sup>a,\*</sup>

<sup>a</sup> Departement of Mechanical Engineering, Faculty of Engineering, Universitas Sriwijaya, Inderalaya 30662, Kabupaten Ogan Ilir, Indonesia

<sup>b</sup> Department of Applied Mechanics and Design, Faculty of Mechanical Engineering, Universiti Teknologi Malaysia, Skudai 81310, Johor, Malaysia <sup>c</sup> Sports Innovation and Technology Centre (SITC), Institute of Human Centred and Engineering (IHCE), Universiti Teknologi Malaysia, Skudai 81310, Johor, Malaysia

\* Corresponding author: hasan\_basri@unsri.ac.id

# ABSTRACT

The objective of this research is to validate the behavior of degradation rate within porous magnesium scaffolds in terms of morphological which includes weight loss after degradation by means of micro-computed tomography ( $\mu$ CT) based on image processing. The main contribution of this work is finding another method to determine morphology based on computer simulation. In the present study, bone scaffold specimens made of pure magnesium that was prepared with three different percentages of porosities 30%, 41%, and 55%. There were immersed and subjected to the dynamic flow rate of simulated body fluid for periods of 24, 48 and 72 hours. One sample of each specimen was scanned by  $\mu$ CT with a resolution of 17  $\mu$ m. The cross-sections of raw data were superimposed by using MIMICS software to form a 3D reconstruction of the samples after degradation. The degradation morphology was collected from the simulation and showed good agreement with the experimental results by only less than 2%. Based on the simulation results, it is possible to give a recommendation for the alternative way in the morphological study of orthopedic applications.

## INTRODUCTION

The most successful strategies in tissue engineering scaffold required that the morphological, micro-architectural, mechanical properties and biodegradation behavior feature well tolerated by the host tissue matching the targeted clinical application in healthcare [1]. It is commonly recognized that morphology actively influences the biological fluids and oxygen transport for their maintenance and subsequently, influencing the in vivo conductive response of the structure after its implantation [2-4]. As a consequence, the degradation properties of scaffolds are relevant both to the biomaterial design and to the long-term success of a tissue engineering structure. Bone tissue engineering scaffold as the biodegradable metals aims to overcome the drawbacks of the current bone regeneration techniques in orthopedic applications. Degradation assessment systems of biodegradable metals must be carefully studied for the specific applications. Therefore biodegradable material selection must be performed because the material must have a good balance between mechanical properties and degradation behavior. Scaffolds must possess enough mechanical properties matching that of the cancellous bone and strength must be maintained for an adequate period of time despite ongoing biodegradation of the implant, which will ultimately lead to scaffold resorption and loss of mass. In the previous work (A.P. Md. Saad et al., 2016) determined the degradation rate of the porous magnesium specimens that was calculated based on weight loss measurement method and hydrogen evolution [5,6].

Micro-computed tomography ( $\mu$ CT) is a technique that can be used to analyze the cancellous and cortical bone micro architecture in micro scale [5]. Microarchitectural analysis of cancellous bone using  $\mu$ CT has been investigated by Müller and Ruegsegger [6]–[9]. Therefore there is a clear potential for  $\mu$ CT techniques to estimate the morphology of tissue engineering bone scaffold in 3D. This paper describes the image processing and three-dimensional visualization of bone scaffold degradation morphology to measure the mass remaining, volume, and weight loss after 72 hours immersion period.

The objective of this research was to develop an image processing method for determination of mass remaining, and weight loss of bone scaffolds. The method provides a valuable alternative to the conventional methods for measurement of weight loss of bone scaffold and can be used to evaluate the bone morphology of medical interest due to the clinical importance of tissue replacement.

In the future, the three-dimensional model based on image processing allows one to investigate the potential connectivity between the host bone and the bone ingrowth within the scaffold.

#### MATERIALS AND METHOD

#### Scaffold preparation and characterization

Cuboid-shaped ( $5 \times 5 \times 3$  mm) of commercially available pure magnesium with a rod diameter of 24.4 mm and 99.9% purity (made by Good fellow Inc, Cambridge, UK) having interconnected holes which were fabricated using CNC machine (HAAS, USA) [5,6]. The samples were drilled using a drill bit of 800 µm in diameter. The porous magnesium of bone scaffold with varying porosity and surface area can be shown in Fig. 1. The morphological of the specimens are shown in Table 1.





Fig. 1. Snapshots of three different morphologies of bone scaffold specimens [5,6]

Table 1. The morphologic details of the bone scaffold specimens [5,6]

Sample	Porosity	Surface Area (mm <sup>2</sup> )	Mass (mg)	Volume (mm <sup>3</sup> )
A	30 %	189.30	82.8	52.87
В	41 %	209.81	70.3	44.57
С	55 %	225.75	53.3	33.83

Three groups of specimens with different morphologies were labeled as sample *A*, *B*, and *C*. The samples were fabricated with three different porosities 30%, 41% and 55% respectively which were selected on the basis of the morphology of cancellous bone [12,13]. For the experimental test, there were 27 porous magnesium samples were prepared. The samples were immersed and subjected to a constant flow rate 0.025 ml/min of simulated body fluid for periods of 24, 48, and 72 hours.

The degradation rate was determined using the weight loss measurement method [5,14]. A diluted acid solution ( $H_2CrO_4$ ) was used to remove the degradation products on the surface of the tested specimens. The weight loss for each dynamic test with different porosities and time immersions was evaluated by using Eq. (1).

$$\Delta W_{\% \, degradation \, rate} = \left[ \left( W_i - W_o \right) / W_i \right] \times 100 \tag{1}$$

Where  $W_i$  is the initial weight and  $W_o$  is the final weight (mg). Weight loss of bone scaffold degradation was quantified and compared to its original weight. For example, 20% weight loss means 80% of the original weight of scaffold remains in the bulk scaffolds, for 100% biodegradation indicates the complete collapse of the scaffold [13].

## Micro-computed tomography (µCT) evaluation

In order to validate degradation rate bone scaffold morphologies, one sample from each porosity (i.e. *A*, *B* and *C*) and each immersion group (i.e. 24, 48, and 72*h*) were scanned by means of microcomputed tomography device. For simulation purposes, there only 9 samples were scanned in the Simulated Body Fluid (SBF). Fig. 3 shows a reconstructed model of 9 immersed samples, from sample *A* = 24*h* to *C* = 72*h*. Raw images with a resolution of 17.20 µm by using a µCT scanner (Skycan 1172, Kontich, Belgium) were taken. The µCT technique has been used in tissue engineering for the purpose to evaluate the tissue integration, tissue formation and scaffold degradation [14]. In general, µCT data sets provide spatial information, suitable for measurements of various bone parameters such as bone volume, bone thickness and bone mineral density [15].

#### Image segmentation and reconstruction

The process to obtain a three-dimensional model from raw  $\mu$ CT images of the bone scaffold is illustrated step-by-step process as shown in Fig. 2. Representative cross-sectional images of the specimen after degradation is shown in Fig 2a. The data from  $\mu$ CT scan was processed by using the so-called gray value thresholding. The most important part of this step is to differentiate between the solid phase (i.e. specimen) from the air. After that, the raw data images are transformed into a binary form or simply black and white voxel and the scale of images have been setup with the true scale. The

white voxel in the micrograph is grouped into the solid phase while a black voxel represents the air. Segmentation or thresholding procedure is conducted by using ImageJ software commands to set the right value of gray scale [16]. ImageJ provides global thresholding algorithms by means of the plugin named Auto Threshold [19-22]. In this study, automatic thresholding algorithm based on the Otsu's method was available in ImageJ is the best method to show good agreement with the interactive method performed by consultant [23-27]. The raw threshold images data has been shown in Fig. 2b and saved in (8-bit TIFF) format images [19]. Finally, the raw data images after segmentation were exported into MIMICS software and the three-dimensional volume rendering is obtained with the 3D mask calculate using software command (see Fig. 2c) on the workstation running 64-bit window XP professional with an Intel Pentium i7 and 128 GB of RAM.



Fig. 2. Illustration of the preparation process of bone scaffold reconstruction: a) Raw data  $\mu$ CT, b) Segmentation of images stacks and c) 3D model reconstruction.

Basically, there are several approaches to the design of the 3D model, include a CAD-based method, image base design, implicit surfaces and space-filling curves [29]. Images-base modeling is the preferred method to provides high quality, more accurate and reliable quantitive 3D data [30]. By using MIMICS command, the volume of virtual models have been obtained and the mass of model is calculated by multiplying the 3D model volume by the Mg material density, as shown in Eq. (2).

$$Mass = V \times \rho \tag{2}$$

Where  $\rho$  is the density of the pure Mg (1.74 g/cm<sup>3</sup>) [31].

#### **RESULTS AND DISCUSSION**

#### **Degradation morphology**

From the raw data set of tomographic images, a three-dimensional reconstruction of bone scaffolds efficiently approximates the real surface which has been generated. Comparison between the simulation and experimental results are shown in Fig. 3.



Fig. 3. Photograph of the comparison morphology between samples and 3D reconstructed models of bone scaffold after biodegradation.

The investigation of the three-dimensional structure leads to further inform the process of implant degraded. A 3D reconstruction of  $\mu CT$  slices using MIMICS allowed a qualitative assessment of the pore interconnectivity within the replica scaffolds.

#### **Simulation results**

The comparison between experimental and simulation results of weight remaining during biodegradation are shown in Table 2. The results showed good agreement with an error less than 2%. The lower of percentage error means that the closer the simulation results are to the experimental results.

**Table 2.** The comparison of bone scaffold weight remaining between experimental and simulation results.

Sample	Periodic immersion (hours)	Weight remaining (%)		
		Experimental	Simulation	% Error
A	24	88.949	89.855	1.010
	48	87.331	88.647	1.485
	72	83.816	85.481	1.947
В	24	86.671	86.828	0.180
	48	78.478	80.142	2.076
	72	78.037	78.620	0.742
С	24	78.912	79.925	1.268
	48	62.946	63.902	1.500
	72	58.705	59.437	1.231

#### Scaffold evaluation

In vitro and simulation biodegradation behavior of magnesium scaffolds (i.e. the percentage of weight loss) was evaluated in SBF for 72 hours and the results are shown in Fig. 4. The results indicate that the scaffold was biodegradable and the degradation of scaffolds was gradually increased over the immersion time. The model's simulation matched the experimental data reasonably well during all immersion periods. As can be seen, the degradation of pure magnesium scaffold was relatively faster than that of the other groups. Sample *C* showed 20% weight loss at 24 hours, but sample A and *B* showed about 10% and 13% weight loss, which indicated that the presence of the porosity significantly enhances the biodegradation rate of the bone scaffolds.

Furthermore, the percentage weight loss of the whole samples after 48 hours of immersion lower than before. For example, the increment weight loss from 48 to 72 hours for *A*, *B*, and *C* samples are

3.5, 1.8, and 4.4% respectively. These results can probably be 3D modeattributed to the presence of gas hydrogen evolution which are difficult to remove from scaffolds surface [5,32]. This may also explain why the biodegradation rate of the scaffold was slower at 72 hours immersion.



Fig. 4. The comparison of the weight loss of bone scaffold after biodegradation between experimental and simulation results.

#### CONCLUSION

In this research, the degradation rate of bone scaffold morphology after degradation has been successfully developed by using computer simulation based on image processing. The simulation results showed good agreement with the experimental results by only less than 2%. The biodegradation behavior of bone scaffold gradually increased under the immersion time and then the presence of the porosity significantly enhances the biodegradation rate of the bone scaffolds.

In the future, the computer simulation based on image processing is introduced to develop a three-dimensional model and it can be easily converted to STL format to analyze mechanobiology of bone scaffolds using finite element analysis and to achieve a threedimensional printing in biomedical applications.

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