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Fabrication and properties of biodegradable ZnO nano-rods/porous Zn scaffolds

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ABSTRACT

Biodegradable ZnO nano-rods/porous Zn scaffolds were successfully fabricated by treating the porous Zn scaffolds in NaCl solution at 80 °C. X-ray diffraction (XRD) analysis shows that the nano-rods *in situ* fabricated on the porous Zn scaffolds in NaCl solution are hexagonal ZnO phase. This result is also confirmed by the image of high resolution transmission electron microscope (HRTEM) and the analysis of X-ray photoelectron spectroscopy (XPS). Compressive tests suggest that the compressive mechanical properties of the ZnO nano-rods/porous zinc scaffolds do not decrease significantly compared with that of the porous Zn scaffolds without treating in NaCl solution. Immersion tests in simulated body fluid (SBF) show that the ZnO nano-rods/porous zinc scaffolds slightly gain in weight after 16 days of immersion. Moreover, these ZnO nano-rods/porous zinc scaffolds can efficiently induce Ca-P precipitation during immersion tests and show a good bioactivity. In addition, antibacterial tests imply that the ZnO nano-rods/porous zinc scaffolds have an excellent antibacterial ability against *Escherichia coli* and *Staphylococcus aureus*. However, the results of cytotoxicity tests of ZnO nano-rods/porous Zn scaffolds are not satisfactory.

1. Introduction

In recent years, metallic zinc and its alloys as new biodegradable metals have attracted considerable attentions of material scientists. Zinc is a highly essential nutrient element for human body and plays an important role in many physiological functions such as normal growth, immune functions, synthesis of DNA polymerase and transcription factors, wound healing, and bone metabolism [1–3]. Zn deficiency will result in a series of detrimental consequences including dystocia, neuropathy, diarrhea, dermatitis, bleeding tendency, hypothermia as well as hypotension [4]. To avoid zinc deficiency, zinc intake is recommended to be 15 mg/day [5]. If the intake levels are greater than the recommended dietary allowance (RDA, for example, 100 mg day^{-1}), the excess zinc can also be tolerated for some time [1,5]. Therefore, zinc is considered to be relatively nontoxic and has a basic safety for biomedical applications [5,6]. In addition, *in vitro* studies imply that osteoblast cell adhesion, proliferation, differentiation (*i.e.*, stimulate bone formation) can be promoted by Zn [7]. Zn is also a highly potent and selective inhibitor of osteoclastic bone resorption *in vitro* [7,8]. More fortunately, Zn has more suitable degradation rate *in vivo* or *in vitro* as implants for biomedical applications compared with Mg and Fe alloys [8–10]. Therefore, Zn and its alloys, being potential biodegradable materials, may be considered to be alternatives to Mg-based or Fe-based implants. For this purpose, ultra-pure Zn [3] and some Zn-based alloys such as Zn-Mg [1,8,11–17], Zn-Ca [8], Zn-Sr [8], Zn-Mg-Ca [4,15], Zn-Mg-Sr [4,15], Zn-Ca-Sr [4], Zn-Ag [18], Zn-Cu [19,20], Zn-Mn [21], Zn-Mn-Cu [22], Zn-Li [23], and Zn-Sn [24] alloys have been developed. Although ultra-pure Zn and these Zn alloys have much lower corrosion rates compared with Mg and its alloys in aqueous solutions, the densities of these materials are much greater than that of natural bone (around 7.14 g/cm^3 *vs.* 1.8–2.1 g/cm^3). To decrease the densities of Zn-based implants, porous scaffolds can be utilized to replace bulk Zn and its alloys. For this reason, some porous Zn-based scaffolds have been fabricated and their mechanical properties, degradation behavior were also investigated [9,25,26].

When a porous scaffold is implanted into human body, bacterial infection that is one of the most serious complications after surgery is an unavoidable question [27–29]. Unfortunately, the antibacterial activity of bulk Zn is not satisfactory [30]. However, ZnO nanomaterials exhibit a prominent and broad-spectrum antibacterial activity and have potential applications as an antimicrobial agent in many fields (such as food packing industry, air filter system, and surgical instruments) [31–34]. Therefore, it can be expected that a porous Zn scaffold with ZnO nanomaterials will have an ideal antimicrobial ability.

In the present work, porous zinc scaffolds as biodegradable implants were prepared by air pressure infiltration method (APIM), and ZnO

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Table 1
Chemical composition of the commercially pure zinc.

Elements	Zn	Pb	Cd	Fe	Cu	Sn	Al
Content (wt%)	Bal.	0.0012	0.0020	0.0008	0.0001	0.0003	0.0004

nano-rods were *in situ* fabricated on the porous Zn scaffolds. The structures and morphologies, mechanical properties, *in vitro* biodegradation, antibacterial activity, and cytotoxicity of the ZnO nano-rods/porous Zn scaffolds were investigated in detail.

2. Materials and Methods

2.1. Fabrication and Pore Structural Characterizations of Porous Zn Scaffolds

Porous zinc scaffolds were fabricated by air pressure infiltration method (APIM) using commercially pure zinc ingot (≥99.995%) and NaCl particles sieved by standard sieves into around 150–300 μm in size as raw materials. The chemical composition of the zinc ingot used is listed in Table 1, and NaCl particles that have irregular shapes are depicted in Fig. 1. The schematic diagram of the APIM setup is presented in Fig. 2. The detailed fabrication process of a porous Zn scaffold can be found in our previous work [9].

The total and open porosities of the obtained porous Zn scaffolds were measured by a hydrostatic weighing method described in Ref. [9]. The apparent densities of the porous Zn scaffolds were also determined by the method used in Ref. [9]. Commercial Image-Pro Plus 6.0 software was employed to statistically determine the pore sizes and the size distribution using SEM images of the porous Zn scaffolds. At least five regions of the scaffolds including transverse and longitudinal sections were calculated.

2.2. Fabrication of ZnO Nano-rods/Porous Zn Scaffolds

To *in situ* fabricate ZnO nano-rods on porous Zn scaffolds, the obtained porous Zn scaffolds were cut into cylindrical specimens having different sizes of φ10 mm × 15 mm or φ10 mm × 3 mm by electrical discharge machining, and then these specimens were treated in 200 g/L NaCl solution at 80 °C for different periods. After that, these specimens were ultrasonically treated in deionized water to leach out the Cl^- ions adhered on the specimens. A solution of 0.1 mol/L $AgNO_3$ was used to test whether the Cl^- ions had been removed completely. Finally, ZnO nano-rods/porous Zn scaffolds were obtained.

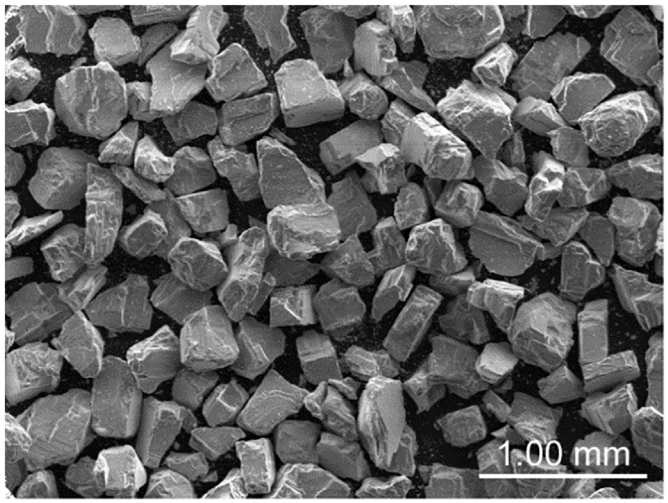

Fig. 1. Morphology of NaCl particles used in fabrication of porous Zn scaffolds.

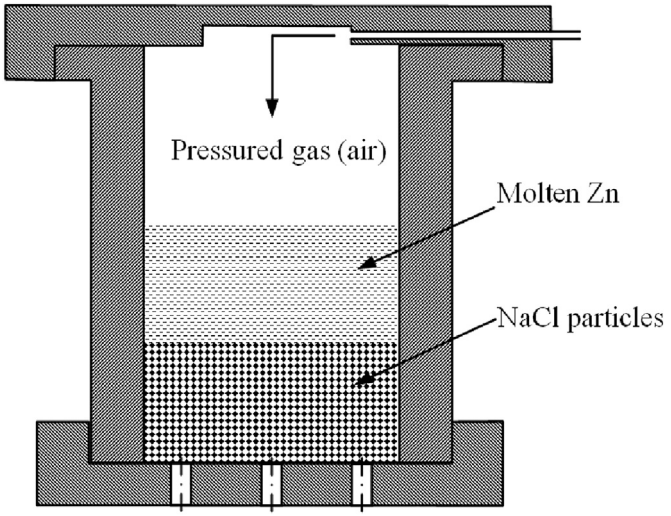

Fig. 2. Schematic diagram of the setup for air pressure infiltration method.

2.3. Compressive Tests

The compressive mechanical properties of the ZnO nano-rods/porous Zn scaffolds were determined by quasi-static uniaxial compressive tests carried out on a WDW-200D testing machine at room temperature. Cylindrical specimens having a size of φ10 mm × 15 mm were used in compressive tests. The tests were performed under displacement control with a crosshead speed of 1 mm/min. Porous Zn scaffolds that were not treated in 200 g/L NaCl solution were also tested as counterparts.

2.4. Immersion Tests

In vitro degradation tests and Ca-P precipitation of the ZnO nano-rods/porous zinc scaffolds were performed in simulated body fluid (SBF). The chemical composition of the SBF was as follows: 8.035 g/L NaCl, 0.355 g/L $NaHCO_3$, 0.225 g/L KCl, 0.231 g/L $K_2HPO_4{\cdot}3H_2O$, 0.311 g/L $MgCl_2{\cdot}6H_2O$, 0.292 g/L $CaCl_2$, and 0.072 g/L Na_2SO_4 [9]. Specimens with a size of φ10 mm × 3 mm were soaked in 150 mL of the SBF remained at 37 °C for different periods. During immersion tests, the pH values of the SBF were measured with a digital pH meter. After immersion, the specimens were removed from the SBF and gently rinsed with deionized water. Then the specimens were dried at room temperature. The weight losses of the specimens were calculated by the following equation:

$$Weight\ loss\,(\%) = \frac{W_i - W_f}{W_i} \times 100\% \quad (1)$$

where W_i and W_f are the weights of a specimen before and after immersion in SBF, respectively. Porous Zn scaffolds that were not treated in 200 g/L NaCl solution were also tested as counterparts. The data of weight losses were statistically analyzed by one-way ANOVA and $p < 0.05$ was considered to be statistically significant.

2.5. Antibacterial Activity Tests

Nutrient solution was prepared by dissolving 33.0 g nutrient agar (Qingdao Hope Bio-technology Co., LTD.) into 1000 mL high purity water. The obtained nutrient solution contains 10.0 g/L peptone, 3.0 g/L beef extract powder, 5.0 g/L sodium chloride, and 15.0 g/L agar. Phosphate buffered saline (PBS) was prepared by dissolving 2.83 g Na_2HPO_4, 1.36 g KH_2PO_4, and 1.0 g Tween-80 into 1000 mL high purity water. The nutrient solution, PBS, and all vessels were sterilized in a pressure steam sterilizer at 121 °C for 20 min.