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Preparation and characterization of nanosilver-doped porous hydroxyapatite scaffolds

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Abstract

Silver nanoparticle (Ag-np) is a new kind of antibacterial agent which is widely used in medical supplies. In this study, a simple approach is described to obtain Ag-doped HA scaffolds. Hydroxyapatite (HA) bone scaffolds with controllable pore size were fabricated by a micro-syringe extrusion system. Sintered HA scaffolds were then immersed in silver reaction hydrosol in order to get Ag-doped HA scaffolds. SEM and EDS shows a uniform distribution of silver nanoparticles on the surface of HA scaffolds. Such Ag-np doped HA scaffolds, displaying a good antibacterial activity against *Escherichia coli* (*E. coli*), are likely to prevent bone implant associated bacterial infections.

Keywords: Silver nanoparticles; Hydroxyapatite; Porous scaffold; Antibacterial activity

1. Introduction

Bacterial infection, which may result in culturing and implantation failure, is a serious threat for tissue engineering [1]. Due to the huge number of transplantation surgeries in bone, infections still have a great influence on morbidity, mortality, and medical costs though bacterial infections rarely occur in the bone tissue transplantation [1–3]. Therefore, antibacterial activity as an additional prevention measure, is necessary in bone transplantation [2,4].

The rising antibiotic resistance has become a major clinical problem because of the long-term use and abuse of antibiotics [5]. Ag-np, as a new generation of antimicrobials, has gained enormous attention [6–8]. Due to their extremely large surface area Ag-nps, compared with larger silver particles, show much stronger inhibition and sterilization effect on dozens of pathogenic microorganisms [9], such as *Escherichia coli* [4,6,8,9], *Staphylococcus aureus* [4,8,10], *Candida albicans* [8] and *Pseudomonas aeruginosa* [10]. Meanwhile, Ag-np does not provoke drug resistance. In this case Ag-np has been widely used in the biomedical field, especially under the trend of increasing resistance of bacteria to antibiotics [2,5,11].

In terms of bone transplantation, Ag-np usually acts as doping material in bone scaffolds. There are some literatures on Ag doping bone scaffolds in the recent years. For instance, Arita Dubnika and Zalite [12] fabricated silver doped porous HA scaffolds using a viscous mass foaming technique and then sintering at different temperatures. The obtained ceramics were characterized as an irregular, open and connected pore system. However, biological evaluation needs to be further studied. Rotimi A. Bakare et al. have synthesized and characterized collagen grafted poly(3-hydroxylbutyrate-co-3-hydroxylvalerate) (PHBV) film for bovine serum albumin (BSA) capped silver (Ag/BSA) nanoparticles [13]. It was reported that the nanoparticles loaded on PHBV film could potentially serve as a scaffold to promote the growth of bone cells while inhibiting bacteria. P. Jiang et al. [14] prepared a series of macroporous–mesoporous bioglasses (MMBGs), doped with Ag, TiO_2 and Ag/TiO_2. Compared with pure MMBGs, much higher antimicrobial efficiency, faster hydroxyapatite-forming ability and better drug sustained release performance were found in MMBGs with Ag,

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TiO_2 or Ag/TiO_2. Similar researches about Ag doped materials for bone scaffolds were also reported [15–17]. Nonetheless, the pores of Ag doped scaffolds mentioned above are mostly uncontrollable.

In this work a method to synthesize silver nanoparticles using oleic acid was developed, and HA scaffolds with controllable pore size were made by a micro-syringe extrusion system. During the process of synthesis, the pure HA scaffolds were immersed in the reaction solution for 4 h to obtain Ag-np doped HA bone scaffolds. Its antibacterial property was evaluated by the Kirby–Bauer method. The antibacterial property of the test specimens was indicated by the size of inhibitory zone formed around the test specimens. A larger zone of inhibition corresponds to a stronger antibacterial property.

2. Materials and methods

2.1. Materials

Commercially available HA powder (needle like particle, 20 nm width and 150 nm length) was supplied by Emperor Nano Material, China. Poly-carboxylic acid ammonium salt (40 wt% solution) was obtained from Shanghai Guben Industrial Co. Ltd. Ammonia water, silver nitrate, oleic acid, hydrazine hydrate, glycerol and some other necessary chemical reagents used in the experiment were purchased from Sinopharm Chemical Reagent Co. Ltd. All materials used in antibacterial testing were provided by School of Life Science and Technology, Huazhong University of Science and Technology.

2.2. Preparation of HA scaffold via micro-syringe extrusion system

HA slurry (30 vol%) was prepared in glycerol(30 wt%)–water solution. 2 wt% poly-carboxylic acid ammonium salt was used as a dispersant to make the slurry much more uniform and stable. Ammonia water (5 mol/L) was used to adjust the pH to 9–10 so that the slurry has a better stability and processability [18]. After the slurry was prepared, it was extruded by the micro-syringe extrusion system. On adjusting the processing parameters, this system can fabricate scaffolds with desired pore size [19]. Then these scaffolds were dried at room temperature for 24 h. More detailed preparing processes can be referred to in Huang Wei, Wu Bin and Wu Quan's studies [19–21]. In order to enhance scaffolds' mechanical property to a relatively high level, a staged sintering process was used. In short, the scaffolds were pre-sintered at 400 °C for 30 min and then sintered at 1200 °C for 30 min using a commercial microwave oven (Hamilab-V1500, SYNOTHERM Co. Ltd, Changsha,China).

The pore size distribution of the sintered scaffolds was measured by a portable USB Digital Microscope (A005+, Shenzhen D&F Co., Ltd). Six sintered HA scaffolds (more than 120 pores in total) have been counted. The phase purity and constitution of the HA scaffolds after sintering were examined by XRD (X'Pert PRO, PANalytical B.V., Netherlands).

2.3. Synthesis of silver nanoparticles

Chemical reduction is a simple way to synthesize small size silver nanoparticles with a high stability [22–24]. In this study, silver nitrate as precursor, oleic acid as stabilizer and hydrazine hydrate as reducing agent were used for the synthesis of silver nanoparticles. In a typical synthesis process, silver nitrate (0.10 g) was added to 20 mL distilled water in a flask. Meanwhile, oleic acid (2.00 g) and hydrazine hydrate (1.50 g) were added to 30 mL distilled water in another flask. Then the above two solution were mixed and stirred with a magnetic stirrer for 4 h at room temperature without any protective gas.

The obtained silver hydrosol was characterized by transmission electron microscopy (TEM) (Tecnai G2 F30 microscope) and ultraviolet–visible spectroscopy (UV–vis) (Cary 50 scan). Silver nanoparticles, centrifuged from silver hydrosol, were characterized by X-ray diffraction (XRD) (X'Pert PRO, PANalytical B.V.).

2.4. Preparation of Ag-np doped HA scaffold

The HA scaffolds were divided into two groups. The first group (called HA-O) was the original scaffolds without further processing, while the second group (called HA-D) was immersed in the reaction solution of silver nanoparticles during the process of synthesis. The morphologies of HA-O and HA-D scaffolds were characterized by SEM. The antibacterial activity of Ag-np doped HA-D scaffolds against *E. coli* (ATCC DH5α) was evaluated by the Kirby–Bauer method, while HA-O scaffold and 10 μL silver hydrosol acted as control samples. 50 μL *E. coli* suspensions were coated on the surface of LB–agar plates (LB liquid media with 2 wt% agar). After that HA-D scaffold, HA-O scaffold and 10 μL silver hydrosol were placed on the plates, followed by incubation of plates at 37 °C for 24 h. Then, the zones of inhibition around the test specimens were observed.

3. Results and discussion

3.1. Properties of HA scaffold

HA scaffolds with controllable pore size can be fabricated by the micro-syringe extrusion system. The pore size of sintered HA scaffolds can be calculated as

$$d = [L-\varphi(1+B)](1-l_s) \quad (1)$$

where d is the expected pore size, L is the distance between two rods, φ is the diameter of nozzle, B is the rate of extrudate swell caused by the Barus effect and l_s is the linear shrinkage of HA scaffolds when sintered at 1200 °C for 30 min. According to our previous research, B is 26.12% [18] and l_s is 31.8% [25]. In this research, L and φ are 800 μm and 350 μm respectively. Hence the calculated size of pore is