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Porous titanium scaffold surfaces modified with silver loaded gelatin microspheres and their antibacterial behavior

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ABSTRACT

Recent years, porous titanium (PT) attracts much attention because of its growing application as medical implants. However, bacterial infections related to implants remain one of the most common and serious complications. In this study, silver loaded gelatin microspheres (Ag/GMSs) were fabricated and incorporated into porous titanium to get antibacterial implants. Prior to incorporating microspheres, oxide film and micro/nanostructures were formed on porous titanium by micro-arc oxidation (MAO) to improve its activity, and the resulted sample is named MPT. Samples were characterized with scanning electron microscope, energy dispersive X-ray spectroscope, transmission electron microscope, X-ray diffractometer, laser scattering particle analyzer and atomic absorption spectrometer. The results showed that Ag^0 particles were loaded to the gelatin microspheres with average diameter of 4.46 μm and Ag/GMSs distributed uniformly on the pore walls of PT and MPT. Ag^0 particles were demonstrated not only formed on the surface of gelatin microspheres but also inside them. The silver loaded samples exhibited a high antibacterial ability against both *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*). The MPT sample was not only better in supporting osteoblast cell viability and good cell proliferation, but also was beneficial to immobilize the silver loaded microspheres to achieve a stronger antibacterial effect than PT. The MPT sample with silver loaded gelatin microspheres increased the cumulative release period of Ag and reached to more than ten days. The main idea of this study also elicited a new surface functionalization strategy for improving antibacterial ability of porous titanium.

1. Introduction

Because of its excellent mechanical properties, corrosion resistance and biocompatibility, titanium becomes an ideal choice for the long-term replacement of hard tissue and has been widely used in biomedical engineering. Porous titanium was introduced due to its superior biocompatibility, adjustable mechanical properties and porous structure [1,2]. Porous structure not only can improve the fixation of solid implants to the surrounding bone tissue by allowing high cell seeding density and tissue in-growth, but also can benefit body fluids being transported through the porous implant [3,4]. However, it is known that titanium implants are generally bioinert. Susceptible surface suffers from problems of interfacial stability with host tissues. So, a bioactive surface with osteoconductive property is required to prevent the formation of fibrous tissue and avoid isolating them from the surrounding bone. Surface modification by micro-arc oxidation (MAO) can alter chemical composition, surface roughness, and morphology of materials [5–9]. Both oxide film and micro/nanostructures formed on PT are in favor of the adhesion, proliferation and osteogenesis of the osteoblast-like cells in vitro [10–13] and enhance osteointegration of Ti implants in vivo [14]. However, another serious dilemma presented for hard tissue replacement materials is lack of antimicrobial property, and deep infection becomes the most common complication. Bacterial infection may occur at the time of surgery or derive from bacteria from remote sources where bacteria are seeded at the vicinities of the implants [15]. Since the impacted bone graft is initially an avascular area, systemic antimicrobial drug cannot easily achieve access to the graft site whereby the risk of postoperative infection is heightened. Local antibiotic delivery has, therefore, become a much-researched area in recent years [16]. The three-dimensional scaffold plays a critical role in the delivery process of growth factors and some drugs. Most of the previous drug delivery works focused on that they can be encapsulated or imbedded within the porous matrices like the three-dimensional scaffold and delivered in a sustained manner to enhance cell growth and morphogenesis, leading to a functionally organized tissue [3,17,18]. Gelatin is a denatured and biodegradable protein obtained by the acid and alkaline processing of collagen. The biosafety of gelatin microspheres has been proven through its long clinical usage in surgical biomaterials and as an ingredient in drugs [19,20]. In recent years, gelatin microspheres (GMSs) are widely employed as a delivery vehicle for the controlled release of biomolecules due to its ability to form polyion complexes with charged therapeutic compounds such as proteins, nucleotides and polysaccharides to prevent rapid release at first and

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realize a subsequent sustained release [21–23]. And it has been used for the controlled release of various kinds of drugs, such as antibiotic drug gentamycin sulfate [24], antihypertensive drugs nifedipine [25], antiasthmatic drug theophylline (THP) [26], anti-inflammatory drugs diclofenac sodium salt [27]and ceftiofur [19].

A large amount of antibiotics like cephalothin, carbenicillin, amoxicillin, cefamandole, tobramycin, and vancomycin have been incorporated in bone implants to form a local release system to prevent post-surgical infections favoring early osteointegration stage [28–30]. However, different drugs have different targets. For example, vancomycin can kill only Gram-positive bacteria and is ineffective against Gram-negative bacteria. However, tobramycin-resistant can be more sensitive on Gram-negative bacteria. This study possibly investigated antibacterial effects of Gram-positive and Gram-negative two kinds of bacteria [31]. Silver has long been known to be a potent antibacterial agent and exhibit more merits than these drugs when doped into implants to overcome the microbial contaminant and infectious disease. For example, silver has the greatest potential against both Gram-positive and Gram-negative bacteria [30,31]. Silver incorporating can efficiently inhibit bacterial attachment onto biomaterials and kill them [32].

In this study, porous titanium scaffolds were treated by the MAO to form the oxide layer and micro/nanostructures. The gelatin mirospheres as silver carrier were incorporated into the porous titanium scaffold uniformly. With the degradation of the gelatin microspheres, silver ions released from the microspheres at a controllable rate. The oxide film layer and micro/nanostructures not only benefit osteoblast attachment and proliferation, but also can provide more favorable conditions for immobilization of the silver loaded microspheres. In this way, antibacterial efficiency increased with higher silver content in the porous titanium and the duration time of antimicrobial was prolonged.

2. Materials and methods

2.1. Preparation of porous titanium (PT)

Porous titanium scaffolds were prepared by powder metallurgy sintering technology. Ammonium hydrogen carbonate (NH_4HCO_3) particles in the range of 200–300 μm were chosen as spacer material. The titanium powders and NH_4HCO_3 particles (1:1) were uniformly mixed and pressed into green compacts under a pressure of 60 MPa. Subsequently, the green compacts were heat-treated at 180 °C for 3 h to volatilize the spacer and form cancellous structure. Finally, the samples were sintered at 1200 °C for 2 h in a vacuum of 1.0×10^{-3} Pa. All of the reagents were of analytical grade in this work including the following experiments.

2.2. Surface modification of porous titanium by micro-arc oxidation

All the prepared PT scaffolds were cleaned with acetone, ethyl alcohol and deionized water, successively. And then, the sample was used as anode and a graphite plate as cathode in an electrolytic bath with 0.1 M H_2SO_4 solution. During this treatment, a DC voltage of 110 V was applied for 3 min. and temperature of the solution was controlled by a cooling system. MAO treatment resulted in the formation of the oxide layer and micro/nano-porous titanium scaffold, namely MPT.

2.3. Synthesis of gelatin microspheres

Gelatin microspheres (GMSs) were prepared by a water-in-oil (w/o) emulsion approach. Briefly, 10 wt.% gelatin (type A) was preheated and then dropwisely added into 60 mL of light liquid paraffin (10:60, *w*/*w*) containing 3% (*w*/*w*) Span-80 at 60 °C under constant stirring for 30 min. When the w/o emulsion system was cooled to 4 °C, glutaraldehyde was added slowly and stirred for 2 h, followed by the addition of 30 mL acetone. The resulted microspheres were washed by ethanol and isopropanol to remove residual light liquid paraffin, collected by filtration and preserved after freeze-drying.

2.4. Preparation of silver loaded gelatin microspheres

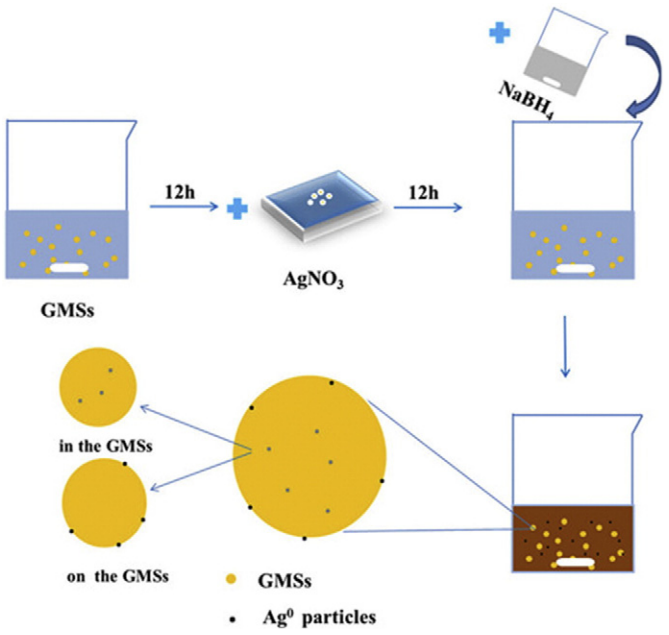
After the prepared GMSs were soaked in silver nitrate solution (10^{-3} M) for 12 h, 4×10^{-3} M sodium borohydride solution was dropwisely added into the solution and aged for 2 h. The silver particles loaded with gelatin microspheres (Ag/GMSs) were washed by centrifugation, and protected from light. The preparation process is shown in Scheme 1.

2.5. Preparation of Ag/GMSs loaded porous titanium

Ag/GMSs were dispersed into ethanol under constant stirring and then the porous titanium samples (PT and MPT) were immersed in the suspension for 12 h. The samples with Ag/GMSs were taken out and cleaned, and noted Ag/GMSs/PT and Ag/GMSs/MPT respectively.

2.6. Characterization of samples

The morphologies of samples were observed by scanning electron microscopy (SEM, JEOL-JSM-700IF). The crystalline structure of PT and the phase components of MPT were analyzed by X-ray diffraction (XRD, Phlips X'Pert PRO) using a Cu Kα radiation in the regular range $2\theta = 20–70°$ at the scanning speed of 5°/min. Particle size analyzer was utilized to measure the particle size of GMSs. X-ray photoelectron spectroscopy (XPS, Kratos XSAM-800, Al Kα radiation) was used to determine the chemical composition and the valence of elements. The microstructure of GMSs was characterized by transmission electron microscopy (TEM, Jeol, Germany). To detect whether silver particles formed inside the network structure of GMSs, Ag/GMSs were embedded in polymethyl methacrylate and sectioned with a diamond band saw (Leica 1600, Germany). The Ag/GMSs section was observed by TEM, and the Ag crystal structure type was additionally verified by careful analysis of high resolution transmission electron micrographs (HR-TEM). The chemical composition was also detected by energy dispersive X-ray spectroscopy (EDS, FEI Quanta 200).



Scheme 1. Preparation scheme of silver loaded gelatin microspheres.