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Novel mesoporous silica-based antibiotic releasing scaffold for bone repair

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Abstract

Tissue engineering scaffolds with a micro- or nanoporous structure and able to deliver special drugs have already been confirmed to be effective in bone repair. In this paper, we first evaluated the biomineralization properties and drug release properties of a novel mesoporous silica–hydroxyapatite composite material (HMS–HA) which was used as drug vehicle and filler for polymer matrices. Biomineralization can offer a credible prediction of bioactivity for the synthetic bone regeneration materials. We found HMS–HA exhibited good apatite deposition properties after being soaked in simulated body fluid (SBF) for 7 days. Drug delivery from HMS–HA particle was in line with Fick's law, and the release process lasted 12 h after an initial burst release with 60% drug release. A novel tissue engineering scaffold with the function of controlled drug delivery was developed, which was based on HMS–HA particles, poly(lactide-co-glycolide) (PLGA) and microspheres sintering techniques. Mechanical testing on compression, degradation behavior, pH-compensation effect and drug delivery behavior of PLGA/HMS–HA microspheres sintered scaffolds were analyzed. Cell toxicity and cell proliferation on the scaffolds was also evaluated. The results indicated that the PLGA/HMS–HA scaffolds could effectively compensate the increased pH values caused by the acidic degradation product of PLGA. The compressive strength and modulus of PLGA/HMS–HA scaffolds were remarkably high compared to pure PLGA scaffold. Drug delivery testing of the PLGA/HMS–HA scaffolds indicated that PLGA slowed gentamycin sulfate (GS) release from HMS–HA particles, and the release lasted for nearly one month. Adding HMS–HA to PLGA scaffolds improved cytocompatibility. The scaffolds demonstrated low cytotoxicity, and supported mesenchymal stem cells growth more effectively than pure PLGA scaffolds. To summarize, the data supports the development of PLGA/HMS–HA scaffolds as potential degradable and drug delivery materials for bone replacement.

Keywords: Mesoporous silica; Poly(lactide-co-glycolide); Antibiotic; Scaffold; Controlled release

1. 1. Introduction

Since the beginning of the 1970s, controlled release technology has experienced great advancement, and motivated more researchers in materials science, chemistry and biomedicine to exert great efforts to develop controlled release systems for different applications [1]. Controlled release systems overcome the disadvantage of traditional drug dosage form, and offer more effective and favorable methods to optimize drug dosage, deliver to specific sites or prolong delivery duration [2]. Nanoparticles [3], mesoporous materials [4], and lipids [5] are the more familiar carriers for delivering genes [6], drugs [7–9] and growth factors [10].

Mesoporous materials, which contain pores with diameter between 2 and 50 nm, have been investigated as a drug

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delivery system since the 1980s [11]. At present, they have been extensively applied in various fields, such as separation [12], catalysis [13], adsorption [14], sensor [15], and photonics [16]. A new application of mesoporous silica – the confinement of a drug or gene in the pores of the material for controlled delivery – was first proposed in 2001 [17], and a wide range of drugs carriers using these kinds of materials have been developed [18,19]. Mesoporous materials have highly organized porous structure with uniform pore size and vast surface area, which make them an excellent candidate as a release carrier.

At present, the most common antibiotic carrier to treat infection after the removal of necrotic bone tissue induced by chronic bacterial osteomyelitis is poly(methylmethacrylate) (PMMA) beads [20–22]. However, these systems must be removed in a second surgical procedure [2]. Herein, we developed new multifunctional composite materials which were produced by hybridizing HMS–HA and PLGA. Gentamycin sulfate (GS), a model antibiotics, was loaded into the HMS–HA particles and then embedded by PLGA spheres for controlled release [23]. Microspheres sintering technique [24,25] was employed to fabricate the PLGA/HMS–HA microspheres scaffold. As a potential drug delivery carrier which can also be used as bone repair material, HMS–HA has the high bioactivity of HA and also inherits the mesoporous structure of HMS. Bioactivity evaluation of the material was done to illuminate its safety and its potential in promoting bone regeneration. HMS–HA particles were used to improve the mechanical properties of PLGA scaffolds, to compensate for the decreased pH values induced by the acidic degradation products of PLGA and to enhance the viability of cells on the scaffolds. On the other hand, PLGA plays an important role in the bonding of HMS–HA particles in the modified scaffold and makes the drug release time longer.

2. Materials and methods

2.1. Materials

Tetraethyl orthosilicate (TEOS), ethyl alcohol (EtOH), $Ca(NO_3)_2$, $(NH_4)_2HPO_4$ and methylene chloride were purchased from Chemical Reagent Factory (Guangzhou, China). Dodecylamine (DDA) was supplied by SSS Reagent (Shanghai, China). Poly(lactic-co-glycolic acid) with a ratio of lactic to glycolic acid monomer units of 50:50 was purchased from Daigang Biomaterials (Jinan, China). This copolymer has an average molecular weight of 31,000 g mol^{-1} with an inherent viscosity of 0.30 dl g^{-1} in chloroform at 30 °C. Gentamycin sulfate (GS) was purchased from Probe (Beijing, China). Poly(vinyl alcohol) (PVA) was obtained from Sigma–Aldrich (Singapore).

2.2. Preparation of HMS–HA–GS composite particles

HMS–HA was synthesized following the method described in Ref. [26]. Briefly, DDA was dissolved in EtOH/deionized water solution (pH 9) containing $Ca(NO_3)_2$, $(NH_4)_2HPO_4$ and NH_4OH. Subsequently, TEOS was added as a silica source and the mixture was stirred at 200 rpm. The reaction mixture conformed to the following molar composition: TEOS: 1.0, DDA: 0.27, EtOH: 9.09, H_2O: 29.6, $Ca(NO_3)_2$:1.0, $(NH_4)_2HPO_4$: 0.6. The mixture was stirred for 1 h, and then aged for 18 h at room temperature. The product was dried at 90 °C, and then the DDA template was removed by EtOH extraction. The nitrogen adsorption/desorption, surface area, and median pore diameter of HMS–HA were measured using a Micromeritics ASAP 2010 M sorptometer.

The HMS–HA–GS particles were achieved by pouring 150 mg dried HMS–HA particles into 100 mg ml^{-1} GS solution. The HMS–HA particles were then soaked in the GS solution for 5 days at 4 °C followed by filtration and drying at 37 °C. The drug-loaded silica particles were weighed again to determine the amount of the loaded drug.

2.3. Fabrication of PLGA microspheres, PLGA/HMS–HA microspheres, and PLGA and PLGA/HMS–HA microspheres sintered scaffolds

PLGA microspheres were prepared using a double emulsion solvent evaporation technique (water/oil/water). GS was dissolved in sodium phosphate buffer (pH 7.2) as the first water phase. Five grams of PLGA was dissolved in 25 ml methylene chloride while the mixture was stirred as the oil phase, and then the first water phase and oil phase were homogenized at 5000 rpm for 30 s with a homogenizer. The emulsion was added dropwise to a 0.5% PVA aqueous solution, and the mixture was stirred for 4 h at 200 rpm, allowing the complete evaporation of the solvent. PLGA microspheres were isolated by vacuum filtration, and washed five times with deionized water.

PLGA/HMS–HA–GS microspheres were prepared using a single emulsion solvent evaporation method. Briefly, 5 g PLGA and HMS–HA–GS particles (1 or 2.5 g) were dissolved in 25 ml methylene chloride, and the mixture was sonicated for 1 min. The resultant mixture was then poured into a 0.5% PVA aqueous solution and stirred for 8 h. PLGA/HMS–HA–GS microspheres were isolated and washed five times with deionized water.

PLGA or PLGA/HMS–HA–GS microsphere sintered scaffolds were fabricated by pouring PLGA microspheres or PLGA/HMS–HA–GS microspheres into cylindrical molds, and then were sintered at 70 °C for 2 h.

2.4. GS encapsulation efficiency of HMS–HA particles and PLGA/HMS–HA microspheres

2.4.1. GS encapsulation efficiency of PLGA/HMS–HA microspheres

Fifty micrograms of PLGA/HMS–HA microspheres were dissolved in 3 ml methylene chloride and centrifuged. The upper solution was collected, leaving out a lower deposit layer. The above process was repeated five times.

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