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An injectable scaffold: rhBMP-2-loaded poly(lactide-co-glycolide)/hydroxyapatite composite microspheres

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ABSTRACT

Poly(lactide-co-glycolide)/hydroxyapatite(50/50) (PLGA/HA(50/50)) composite microspheres were fabricated and treated with a mixture of 0.25 M NaOH aqueous solution and ethanol (v/v = 1/1) at 37 °C. The properties of untreated and treated PLGA/HA(50/50) composite microspheres were determined and compared. The results showed that the surface roughness, HA content and hydrophilicity of the treated PLGA/HA(50/50) composite microspheres increased with treatment time. However, the treatment time should be kept within 2 h in order to maintain the shape of the PLGA/HA(50/50) microspheres. At the same time, a degradation study showed that both the untreated and treated microspheres degraded gradually with time, with the treated microspheres degrading faster in the first 4 weeks. The rhBMP-2-loaded PLGA/HA(50/50) composite microspheres were prepared by solution dipping treated PLGA/HA(50/50) composite microspheres. Mouse OCT-1 osteoblast-like cells were cultured on the untreated, treated and rhBMP-2-loaded PLGA/HA(50/50) composite microspheres and the cell affinity of the various microspheres was assessed and compared. It was found that the surface-treated PLGA/HA(50/50) composite microspheres clearly promoted osteoblast attachment, proliferation and alkaline phosphatase activity. It was considered that the hydrophilicity, osteoconductivity and surface roughness were increased by the increase in the HA component, which facilitated cell growth. Moreover, the rhBMP-2 loaded on the treated PLGA/HA(50/50) composite microspheres could be slowly released and further enhanced osteoblast differentiation. The good cell affinity and enhanced osteogenic potential of the rhBMP-2-loaded PLGA/HA composite microspheres indicate that they could be used as an injectable scaffold.

1. Introduction

Bone grafts, including autografts, allografts and xenografts, have been widely used for repairing bone defects originated from trauma, tumor resection, bone fractures, infections and skeletal abnormities [1–3]. Although some of them are effective for bone regeneration, many defects, such as limited availability, immune reaction, transfer of pathogens and non-biodegradability, have limited the development of bone grafts [4,5]. Bone tissue engineering using osteogenic cells, osteoinductive growth factors and scaffolds alone or in combination appears to be the most promising alternative to existing therapies for bone repair and regeneration [6]. One of the most significant challenges of this technique is to design and fabricate suitable biodegradable scaffolds that can support cell adhesion, growth, proliferation and differentiation, and guide the process of tissue formation.

Recently, the development of scaffolds for tissue engineering has focused on the design and fabrication of biomimetic scaffold materials that can interact with surrounding tissues by biomolecular recognition. Biomimetic material is designed to elicit specific cellular responses and directly form new tissue through some specific interaction. Hydroxyapatite (HA) ($Ca_{10}(PO_4)_6(OH)_2$), as a bioceramic, is an effective component for biomimetic materials since its chemical and structural characteristics are similar to the mineral phase of native bone [7]. Because of its good biocompatibility and osteoconductivity, HA has been widely used as a bone-filling material in dental and orthopedic surgery [8,9]. However, drawbacks such as difficulty of shaping, poor mechanical strength, brittleness and slow degradation rate still limit the direct application of HA as a scaffold for bone tissue engineering [10–12]. It is hoped that composite scaffolds of HA and other biomaterials can overcome the defects mentioned above [13–15].

Much attention has also been paid recently to composites of HA and synthetic polylactone-type biodegradable polymers, such as poly(L-lactide), polyglycolide and their copolymer poly(lactide-co-glycolide) (PLGA), since the polymers possess good mechanical properties, low immunogenicity and toxicity, and an adjustable degradation rate. The combination of HA and polylactone-type polymer can be expected to combine the best features of both materials to obtain the optimum scaffold for bone tissue engineering, since it possesses fundamental characteristics such as bioactiv-

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ity, biomechanical similarity, processability and biodegradability. It can also reduce the acidity of the degradation products of the polylactone-type polymers [16].

Most polylactone/HA composite scaffolds have been fabricated into films, rods, plates, blocks and foam by the solvent evaporation method [17], the electrospinning method[18,19], the solvent casting and particulate leaching (SC/PL) method [20,21], the compression molding method [22,23], the phase separation method [24,25], the gas foaming and particulate leaching (GF/PL) method [26,27], the indirect solid free form (SFF) method [28], etc. Although the geometries and configurations of pre-shaped polylactone/HA composite scaffolds can be continually improved with advancements in the fabrication method, it is still difficult to produce the complicated shapes of scaffolds required to meet clinical needs. Considering that microspheres exhibit flowability and can be injected as a three-dimensional scaffold into various shaped bone defects for bone repair, polylactone/HA composite microsphere-type scaffolds could provide more versatile applications than pre-shaped scaffolds. On the other hand, microsphere-type scaffolds can also perform tissue repair and gene therapy when loaded with special growth factors or drugs. Microsphere-type scaffolds also possess other advantages, such as needing only a minor incision and a more convenient operation for the scaffold transplantation. The polylactone/HA composite microspheres have generally been fabricated by an emulsion–solvent evaporation method and studied as drug delivery vehicles [29,30]. However, a few studies have reported on the application of polylactone/HA composite microspheres as injectable scaffolds in tissue engineering.

On the other hand, although it has been reported that HA can enhance osteoblast growth and differentiation, whether HA can induce bone formation by itself it still a matter of contention. Ono et al. [31] thought HA could not induce bone formation by itself, particularly in sites where bone does not form normally. However, Habibovic et al. [32] indicated the osteoinductive properties of HA in ectopic sites. Therefore, it is necessary to combine the polylactone/HA composite microspheres with another bone inductor to promote and expedite bone formation.

Bone morphogenetic proteins (BMPs) are potent bone inductors, which control osteogenesis. They play a crucial role in cell growth and differentiation in a variety of cell types, including osteoblasts [33]. To improve the efficiency of administering BMPs, the combination of BMPs with biomaterials has received considerable attention [34–40]. BMPs are combined on biomaterials mainly by physisorption [35,37], electrostatic interaction [39] or covalent binding [40]. Of the BMPs, BMP-2 is most extensively researched, and has been used in clinical applications since it is readily available and has a very strong osteoinductive activity [41,42]. It has been reported that BMP-2 can promote the maturation of committed cells to become more differentiated osteoblasts and induce bone formation in ectopic and orthotopic sites in vivo [33,43,44].

The aim of this study was to develop a BMP-2-loaded polylactone/HA composite microsphere-type scaffold as an injectable bone tissue engineering scaffold. First, the PLGA/HA(50/50) composite microspheres were treated with a mixture of NaOH aqueous solution and ethanol. The morphology and properties of the treated PLGA/HA(50/50) composite microspheres were investigated by scanning electron microscopy (SEM), energy-dispersive spectroscopy (EDS), gel permeation chromatography (GPC) and water uptake. At the same time, the degradation of untreated and treated microspheres was studied and compared. The treated PLGA/HA(50/50) composite microspheres were also combined with recombinant human bone morphogenetic protein-2 (rhBMP-2). Finally, adhesion, proliferation and differentiation of cells on the rhBMP-2-loaded PLGA/HA(50/50) composite microspheres were evaluated and compared with that on untreated PLGA/HA(50/50) and treated PLGA/HA(50/50) composite microspheres using the mouse OCT-1 osteoblast-like cell as a model cell in vitro.

2. Materials and methods

2.1. Materials

Glycolide and lactide were purchased from Acros Chemica, N.V. and purified twice by recrystallization from ethyl acetate. Stannous octoate (Sigma, A.R.) was used without further purification. Ethyl acetate was dried by P_2O_5 overnight and then distilled. Hydroxyapatite (4.86 μm of average particle size) was purchased from Sinopharm Chemical Reagent Co., Ltd., China. rhBMP-2 was produced by the Fourth Military Medical University, China. Poly(vinyl alcohol) (PVA, average M_n = 77,000, 87–89% hydrolyzed) was purchased from Tianjin Zongheng Chemical Company, China. Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Invitrogen Corporation, America. Trypsin and ethylenediaminetetraacetic acid (EDTA) were obtained from Sigma. The protein assay kit and alkaline phosphatase (ALP) kit were from Nanjing Jianchen Bioengineering Institute, China.

2.2. Synthesis of PLGA

PLGA (molar ratio of lactide/glycolide = 70/30, M_w = 112,000) was synthesized from L-lactide and glycolide under high vacuum in the presence of stannous octoate as a catalyst (0.05 wt.%) at 160 °C for 20 h according to the literature [45].

2.3. Fabrication of PLGA/HA(50/50) composite microspheres

PLGA/HA(50/50) composite microspheres were fabricated by an emulsion–solvent evaporation method. Briefly, 200 mg of PLGA was completely dissolved in 8 ml of dichloromethane and 200 mg of HA was added to the PLGA solution and stirred thoroughly to form a well-dispersed mixture. The mixture was then emulsified in a PTFE tube by ultrasonication under 300 W of output for 30 s. The formed solid-in-oil emulsion was subsequently dropped into 200 ml of external PVA aqueous solution (1% (w/v) concentration) and further stirred at 500–600 rpm at room temperature for 2–3 h to evaporate the organic solvent. Finally, the formed microspheres were collected by centrifugation, washed five times with distilled water and freeze-dried using a lyophilizer (ALPHA 1–2 LD) to sublimate the remained water and obtain free-flowing PLGA/HA(50/50) microspheres. The average diameter of the PLGA/HA(50/50) composite microspheres was in a range of 50–120 μm. The dried microspheres were stored at 4 °C before used.

2.4. Surface treatment

In accordance with the previously reported method [46], the PLGA/HA(50/50) composite microspheres were immersed in a mixture of 0.25 M NaOH aqueous solution and ethanol (v/v = 1/1) for a predetermined period (0.5, 1, 2 and 3 h) of incubation at 37 °C, then rinsed with deionized water (3 × 10 min) and freeze-dried using a lyophilizer.

The untreated PLGA/HA(50/50) composite microspheres, and the PLGA/HA(50/50) composite microspheres treated with the mixture of 0.25 M NaOH aqueous solution and ethanol (v/v = 1/1) for 1 and 2 h at 37 °C were abbreviated as UT-PLGA/HA, T1-PLGA/HA and T2-PLGA/HA, respectively.

2.5. Characterization of surface property

After various PLGA/HA(50/50) composite microspheres were dried and sputter-coated with gold, their surface morphology them