Electrospun magnetic poly(L-lactide) (PLLA) nanofibers by incorporating PLLA-stabilized Fe_3O_4 nanoparticles

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A B S T R A C T

Magnetic poly(L-lactide) (PLLA)/Fe_3O_4 composite nanofibers were prepared with the purpose to develop a substrate for bone regeneration. To increase the dispersibility of Fe_3O_4 nanoparticles (NPs) in the PLLA matrix, a modified chemical co-precipitation method was applied to synthesize Fe_3O_4 NPs in the presence of PLLA. Trifluoroethanol (TFE) was used as the co-solvent for all the reagents, including Fe(II) and Fe(III) salts, sodium hydroxide, and PLLA. The co-precipitated Fe_3O_4 NPs were surface-coated with PLLA and demonstrated good dispersibility in a PLLA/TFE solution. The composite nanofiber electrospun from the solution displayed a homogeneous distribution of Fe_3O_4 NPs along the fibers using various contents of Fe_3O_4 NPs. X-ray diffractometer (XRD) and vibration sample magnetization (VSM) analysis confirmed that the co-precipitation process had minor adverse effects on the crystal structure and saturation magnetization (Ms) of Fe_3O_4 NPs. The resulting PLLA/Fe_3O_4 composite nanofibers showed paramagnetic properties with Ms directly related to the Fe_3O_4 NP concentration. The cytotoxicity of the magnetic composite nanofibers was determined using in vitro culture of osteoblasts (MC3T3-E1) in extracts and co-culture on nanofibrous matrixes. The PLLA/Fe_3O_4 composite nanofibers did not show significant cytotoxicity in comparison with pure PLLA nanofibers. On the contrary, they demonstrated enhanced effects on cell attachment and proliferation with Fe_3O_4 NP incorporation. The results suggested that this modified chemical co-precipitation method might be a universal way to produce magnetic biodegradable polyester substrates containing well-dispersed Fe_3O_4 NPs. This new strategy opens an opportunity to fabricate various kinds of magnetic polymeric substrates for bone tissue regeneration.

1. Introduction

Bone injuries and defects remain a significant problem in clinical therapy. Compared to conventional therapies, bone tissue engineering offers a new and promising approach for bone repair and regeneration, and has developed rapidly in recent years [1]. Typically, tissue engineering was performed using porous scaffolds in combination with tissue cells and relative factors to assist cell adhesion, proliferation and differentiation. In bone tissue engineering, scaffolds should be osteogeneous and able to provide differentiation cues to stimulate osteoprogenitor or stem cells into an osteogenic phenotype [2]. To this end, an effective way is to mimic the morphological traits, chemical composition and mechanical function of the native bone extracellular matrix (ECM) [3].

Concerning the collagen nanofibrous networks in the native ECM, a strategy to design biomimetic nanofibrous scaffolds has attracted considerable attention in recent years [4,5]. Due to the simplicity, diversity, and adjustability, electrospinning has developed into a commonly used method to fabricate nanofibers [6]. In addition to the beneficial features of the nanofibrous structure, other biological cues can be endowed to nanofibrous scaffolds to regulate cell behavior. Strategies such as the incorporation of bone-bioactive inorganic components, like calcium phosphate compounds [7], or involvement of osteogenic stimulatory signals, like growth factors and genes [8], have been verified as effective approaches to achieve the ultimate goal of bone reconstruction.

Studies have shown that static magnetic fields can alter cell proliferation, migration, and orientation [9–12], and may be one beneficial factor for enhancing bone tissue regeneration. It was demonstrated that magnetic fields were capable of preventing decreases in bone density [13]. Exposing bone wounds to magnetic fields can not only accelerate bone fracture healing [14], but also have a stimulating effect on the microstructure and the mineralization process during bone repair [15]. There is also substantial evidence indicating that magnetic fields are able to template mineral deposition during the early stages of the biomineralization process when osteoblasts were cultured in vitro [16].

In view of the advantageous features of both the nanofibrous networks and the magnetic fields, researchers have begun to combine magnetic components into biodegradable nanofibers to produce paramagnetic nanofibrous composite scaffolds [17,18]. To fabricate these composite nanofibers, a commonly used method is to mix dry

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inorganic powders with polymeric solutions followed by subsequent electrospinning [17–19]. One major difficulty in preparing such nanocomposites is the inability to obtain stable dispersions of the magnetic nanoparticles (NPs) in polymer solution. To ameliorate the dispersibility of NPs in polymeric matrices, several surface treatments had been applied including silanization, [20,21] polymer brushes coating, [22,23] grafting, [24,25] etc. To ensure the dispersion of Fe_3O_4 NPs in water or organic solvents, sodium citrate, polyacid or oleic acid have also been applied. However, the dispersion of Fe_3O_4 NPs in polymeric matrices was still far from satisfactory due to their incompatible interfaces. Using a chemical co-precipitation method, Fe_3O_4 NPs have been successfully incorporated into poly(vinyl alcohol) (PVA) nanofibers by combining an in-situ composite method with electrospinning technology [26]. In this process, Fe_3O_4 NPs were prepared and co-precipitated and stabilized in the presence of PVA to avoid agglomeration.

Among all polymeric materials, biodegradable lactide-based polyesters are the most extensively used biomaterials in tissue engineering [27]. Therefore, a magnetic poly(L-lactide) (PLLA)/Fe_3O_4 nanofibrous scaffold was prepared in this study. For the first time, to the best of our knowledge, a novel method of co-precipitating Fe_3O_4 NPs in the presence of PLLA has been developed to stabilize Fe_3O_4 NPs using PLLA directly. Subsequently, PLLA/Fe_3O_4 nanofibrous scaffolds were electrospun from PLLA solutions containing the PLLA-stabilized Fe_3O_4 (Fe_3O_4@PLLA) NPs. The morphological characteristics and the magnetic properties of the obtained composite nanofibers were evaluated. In addition, their cytotoxicity and cell affinity were assessed by in vitro culture with calvaria-derived cells (MC3T3-E1) in extracts or directly on the magnetic composite nanofibers containing different amounts of Fe_3O_4@PLLA NPs.

2. Materials and methods

2.1. Materials

Poly(L-lactide) (PLLA, Mw = 100,000) and 2,2,2-trifluoroethanol (TFE, 99%) were purchased from Sigma-Aldrich for electrospinning, and used without any treatment or further purification. Iron (II) chloride tetrahydrate, iron (III) chloride hexahydrate, and sodium hydroxide were all obtained from Tianjin Kermel Chem. Co., Ltd. (China) and used directly for the preparation of Fe_3O_4 NPs. All other reagents and solvents used were of analytical grade and supplied by Beijing Chemical Reagent Co., Ltd. (China).

2.2. In-situ formation of Fe_3O_4@PLLA NPs

A modified chemical co-precipitation method was proposed to form Fe_3O_4@PLLA NPs in-situ. [26] Briefly, PLLA was dissolved in TFE (1 mg/mL) overnight at room temperature. The solution was bubbled with nitrogen gas for 30 min before sodium hydroxide was added. To the transparent solution, an aqueous solution containing Fe(II) (0.124 mmol/m) and Fe(III) (0.287 mmol/m) salts was added dropwise. Within several minutes, the color of the mixture changed from yellow to black, indicating the completion of the reaction. Then, deionized water (with oxygen removed by nitrogen bubbling) was added into the system to co-precipitate the Fe_3O_4@PLLA NPs. The precipitates were collected by centrifugation, washed three times with deionized water, and lyophilized. For comparison, pure Fe_3O_4 NPs were prepared in a similar way without the addition of PLLA and the co-precipitation.

2.3. Electrospinning of PLLA/Fe_3O_4 composite nanofibers

Fe_3O_4@PLLA NPs were added to a PLLA/TFE solution (10%w/v) at different concentrations (Fe_3O_4@PLLA NPs to PLLA: 0/100, 2.5/100, 5/100, 7.5/100 and 10/100 in w/w, which were named as PLLA/Fe_3O_4(0), PLLA/Fe_3O_4(2.5), PLLA/Fe_3O_4(5), PLLA/Fe_3O_4(7.5) and PLLA/

Fe_3O_4(10), respectively). Ultrasonication (250 W) was applied to help the dispersion of Fe_3O_4@PLLA NPs in the solution before electrospinning. Each of the mixed solutions was placed into a 10 mL syringe equipped with a stainless steel gauge needle (inner diameter 1.2 mm). The needle was connected to a high voltage power supply (DW-P403-1ACCC), and the grounded counter electrode was attached to flat aluminum foil, which was also used as the collector. The electrospinning parameters were set as: applied voltage 15 kV, receiving distance 15 cm and flow rate 0.5 mL/h. The obtained fibers were then dried rigorously in a vacuum oven for one week at room temperature for further use.

2.4. Characterization

Fourier transform infrared spectra (FTIR) were recorded using a FTIR spectroscope (Nicolet 6700, USA) with the wavenumber ranging from 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1}. Crystal and chemical structures were evaluated by a X-ray diffractometer (XRD, D/Max 2500VB2+, Rigaku, Japan) with a fixed incidence of 1° at a 2θ scanning rate of 10°/min in the range of 5–90° using CuKα radiation with a monochromator. Morphological observations were conducted by a scanning electron microscope (SEM, Supra 55, Zeiss, German) at an accelerating voltage of 20 kV after being sputter-coated with platinum (30 mA, 60 s) using a sputter coater (Polaron E5600, USA). The dispersibility of Fe_3O_4@PLLA NPs in TFE and in PLLA was detected using a transmission electron microscope (TEM, Hitachi, H-800) and iron mapping illustration. The mapping illustration was performed under the same parameters to SEM observation and the exposure time was 180 s. Particle sizes and size distribution were obtained by averaging 200 particle sizes using Image J image visualization software (National Institutes of Health, USA) based on the obtained TEM images. To prepare the samples for TEM observation, NPs were dispersed in TFE for 24 h before being dropped onto copper grids. Nanofibers were then directly mounted onto carbon-coated copper grids during electrospinning. M–H curves of pure Fe_3O_4 NPs, Fe_3O_4@PLLA NPs, and the PLLA/Fe_3O_4 composite nanofibers were evaluated by employing vibration sample magnetization (VSM, JDAM-2000) at room temperature.

2.5. Biological property evaluation

2.5.1. Cell culture and cell seeding

MC3T3-E1, a mouse calvaria-derived cell line, was purchased from Cell Culture Center, Peking Union Medical College (China). Cells were cultured in a Dulbecco's modified Eagle's medium (DMEM, Hyclone) supplemented with 10% fetal bovine serum (FBS, PAA, Germany), 100 IU/mL penicillin (Sigma), and 100 mg/mL streptomycin (Sigma) in an incubator (Sanyo, Japan) with 5% CO_2 at 37 °C and saturated humidity. Once the cells reached 80% confluency, the MC3T3-E1 cells were digested by 0.25% trypsin (Sigma) and 0.02% ethylene diamine tetraacetic acid for further use. Before cell seeding, pure PLLA and PLLA/Fe_3O_4 composite nanofibrous scaffolds were sterilized with ultraviolet (UV) light for 2 h and then immersed in 70% ethanol for 10 min, followed by rinsing three times with phosphate-buffered saline (PBS) solution.

2.5.2. Extract toxicity assay of PLLA/Fe3O4 nanofibers

According to ISO 10993-12: 200, various PLLA/Fe_3O_4 composite nanofibers containing different amounts of Fe_3O_4 NPs were soaked in DMEM for 24 h, and the extracts were used for cell culture. The cell culture was carried out in 96-well plates with 20 μl of cell suspension (2.5×10^5 cell/mL) and 180 μl of extracts added into each well, and cultured for 7 days. The cell proliferation rates were tested by Cell Counting Kit-8 (CCK-8, Beyotime, China). CCK-8 is a kind of yellow solution that can be reduced to orange by active cells, whose absorbance is directly proportional to cell number. At each predetermined