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Magnetic nanoparticle-loaded electrospun polymeric nanofibers for tissue engineering

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ABSTRACT

Magnetic nanoparticles have been one of the most attractive nanomaterials for various biomedical applications including magnetic resonance imaging (MRI), diagnostic contrast enhancement, magnetic cell separation, and targeted drug delivery. Three-dimensional (3-D) fibrous scaffolds have broad application prospects in the biomedical field, such as drug delivery and tissue engineering. In this work, a novel three-dimensional composite membrane composed of the tri-block copolymer poly(ε-caprolactone)-poly(ethylene glycol)-poly(ε-caprolactone) (PCL-PEG-PCL, PCEC) and magnetic iron oxide nanoparticles (Fe_3O_4 NPs) were fabricated using electrospinning technology. The physico-chemical properties of the PCEC/Fe_3O_4 membranes were investigated by Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD) and differential scanning calorimetry (DSC). Morphological observation using scanning electron microscopy (SEM) showed that the composite fibers containing 5% Fe_3O_4 nanoparticles had a diameter of 250 nm. In vitro cell culture of NIH 3T3 cells on the PCEC/Fe_3O_4 membranes showed that the PCEC/Fe_3O_4 fibers might be a suitable scaffold for cell adhesion. Moreover, MTT analysis also demonstrated that the membranes possessed lower cytotoxicity. Therefore, this study revealed that the magnetic PCEC/Fe_3O_4 fibers might have great potential for using in skin tissue engineering.

1. Introduction

Three-dimensional (3D) fibrous scaffolds have many applications in the biomedical field, including systems for drug delivery and tissue engineering. In particular, electrospun fibrous scaffolds have attracted much attention in the past decades as an ideal carrier or substitution for applications in drug delivery and tissue engineering [1–3]. Considering use in drug delivery, many drugs can be adsorbed on the surface of electrospun scaffolds or encapsulated into scaffold fibers: the drugs are successively released through diffusion or through the degradation of the scaffold matrix [4,5]. In tissue engineering, 3D fibrous scaffolds are generally used as substitutes to reconstruct the lost functions of diseased or injured tissues [6,7]. To improve the remediation effect, a large number of therapeutic agents such as drugs, cells, or functional nanoparticles are integrated into the scaffold to form a structure with unique functions.

Electrospun scaffolds have prominent advantages in uses as implants for tissue engineering applications, due to their special characteristics [1,8,9]. The electrospun fibrous scaffolds are made of a mass of continuous submicrometer fibers and/or nano-sized fibers. Because of this, their morphology is very similar to that of the fibrillar structure of the extracellular matrix (ECM); hence, they can provide mechanical support for cell attachment and growth [4,10,11]. Up to now, a variety of degradable polymers have been employed to make various fibrous mats, and used in tissue engineering [7,9,12].

The scaffold matrix has an obvious effect on its properties; further to this, however, the characteristics and the type of therapeutic agents or fillers also play a vital role in endowing the functions of scaffolds. Curcumin-loaded fibrous scaffolds, for instance, have an obvious antitumor efficacy and can also enhance dermal healing [13]. Cell co-cultured scaffolds may be used for tissue reconstruction [14,15]. If calcium phosphate salts such as hydroxyapatite nanoparticles (n-HA) or tertiary calcium phosphate (TCP) are used as fillers, the composite scaffolds might be applied for guiding bone tissue regeneration [16,17]. If silver

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nanoparticles are added, the scaffold may have anti-bacterial function [18,19]. Therefore, the characteristic of incorporated therapeutic agents or fillers greatly influences the functions and applications of composite scaffolds.

Metallic oxide nanoparticles such as iron oxide nanoparticles (Fe_3O_4), have been successfully used as fillers to prepare various kinds of multi-functional nano-materials in biomedical fields, due to their unique magnetic responsiveness [20,21]. Fe_3O_4 nanoparticles have found definite applications in biomedical fields, including clinically diagnostic MRI and biosensing systems; they have also been used as nanovehicles for targeted delivery of various therapeutic agents [22,23]. Huang et al. found that iron oxide nanoparticles embedded magnetic fibers have an efficient heating capacity when exposed to an alternating magnetic field. Magnetic field induced hyperthermia seemed much more efficient in killing of ovarian cancer cells than water bath heating [24]. In our published work, we have confirmed that curcumin-loaded PCEC scaffold had a good therapeutic efficacy in accelerating dermal wound healing [13]. PCEC is a biodegradable tri-block copolymer which can be simply prepared by a one-step reaction using ε-CL and PEG. According to application ranges, its physical-chemical properties such as molecular weight, degradation rate, surface wettability and mechanical strength could be tailored by adjusting the feed ratios of ε-CL/PEG. Therefore, we plan to develop a therapeutic scaffold using PCEC as matrix for preparation of a dermal dressing with dual functions of preventing tumor recurrence and improving dermal wound healing after an excision of malignant tumor in the skin. As a part of whole plan, in this study, we will firstly prepare a magnetic fibrous scaffold by electrospinning and investigate its physico-chemical properties and cell biocompatibility in vitro.

2. Materials and methods

2.1. Materials

The tri-block poly(ε-caprolactone)-poly(ethylene glycol)-poly(ε-caprolactone) (PCL-PEG-PCL, PCEC) copolymer used in this study was prepared through a ring-opening polymerization of ε-caprolactone (ε-CL) initiated by poly(ethylene glycol) (PEG, Mn = 4.0×10^3) following a protocol previously developed [25]. Briefly, 0.4 g of PEG (0.1 mmol), 9.6 g of ε-caprolactone (0.0842 mol), and 0.05 g of $Sn(Oct)_2$ (0.1 mmol) were introduced into a dry three-necked flask under a protective nitrogen atmosphere. The polymerization was performed at 130 °C under mechanical stirring. After a reaction of 5 h, the system underwent degassing for a further hour. When the bottle was naturally cooled to room temperature, the resulting PCEC copolymer was dissolved in dichloromethane and precipitated in pre-cooled petroleum ether, then vacuum-dried until a constant weight was obtained. The molecule weight (*M*w) of the prepared PCEC copolymer, previously determined with gel permeation chromatography, was 6.2×10^4 g/mol [26].

The Fe_3O_4 powder used in this study was prepared through a thermal decomposition method reported in literature [27]. Briefly, 2.12 g of $Fe(acac)_3$ (6 mmol) was firstly added into a mixture of 30 mL oleylamine and 30 mL benzyl ether. Then the mixed $Fe(acac)_3$ solution was dehydrated at 110 °C for 1 h under N_2 atmosphere. After that, it was quickly heated to 300 °C and kept at this temperature for 1 h. When the reaction was completed, the mixture was naturally cooled down to room temperature. The produced Fe_3O_4 NPs were precipitated in 100 mL of ethanol, followed by centrifuging. After alternate washing with hexane and ethanol (three times), the Fe_3O_4 NPs were vacuum-dried to a constant weight at 50 °C, and then stored in an air-tight bag for further use.

Other chemical agents including hexane, CH_2Cl_2, and benzyl ether were purchased from Chengdu Kelong Chemical Co., Ltd. (Chengdu, China). All chemicals were used directly without further purification.

2.2. Electrospinning

Firstly, 1.0 g of dried PCEC copolymer was dissolved in 20 mL of CH_2Cl_2 (PCEC solution concentration 50 mg/mL). 100 mg of dried Fe_3O_4 NPs was re-dispersed in 1 mL hexane to form a uniform solution through stirring and ultrasound sonication. When preparing the magnetic fibers, a measured volume of PCEC solution was evenly mixed with a certain volume of the Fe_3O_4/hexane solution under stirring, to obtain a predetermined Fe_3O_4 content.

For example, to prepare a PCEC/Fe_3O_4 composite membrane containing 10% (w/w) Fe_3O_4 NPs, 1 mL of Fe_3O_4/hexane solution was added dropwise into 18 mL of PCEC/CH_2Cl_2 solution under stirring. Then the mixed solution was placed into a 20-mL plastic syringe (BD) which was fixed on a micro-injection pump. The electrospinning was carried out at room temperature with a 18 kV high-voltage power. The flow rate of spinning solution was adjusted to 6 mL/h. The space between the spinneret and the aluminum foil collector was set to 12 cm. The resulting PCEC/Fe_3O_4 composite membranes were collected from alumina foil collector, and then dried at 40 °C for 48 h, to remove any residual solvent.

2.3. Characterization of PCEC/Fe_3O_4 membranes

2.3.1. Fourier transform infrared Spectrum (FT-IR)

Fourier transform infrared (FT-IR) analysis of Fe_3O_4, pure PCEC, and PCEC/Fe_3O_4 mats with 10% Fe_3O_4 were carried out using a Nicolet 6700 FTIR spectrometer (Thermo Electron Corp., Madison, Wis., USA). Transmission infrared spectra were recorded in the range of 400–4000 cm^{-1}.

2.3.2. XRD analysis

XRD analysis of Fe_3O_4, PCEC, and PCEC/Fe_3O_4 mats with 10% Fe_3O_4 were carried out on a Panalytical X' Pert Pro MPD DY1291 (PHILIPS, Netherlands) diffractometer. The specimens were tested in a 2θ range of 10° to 70° at a scanning rate of 4°/min.

2.3.3. Transmission electron microscopy (TEM)

For TEM analysis, Fe_3O_4 powder was observed on an FEI Tecnai 20 transmission electron microscope (FEI Co., Hillsboro, Oregon, USA) at 120 kV, while PCEC/Fe_3O_4 fibers were analyzed with a HITACHI H-600 electron microscope. The PCEC/Fe_3O_4 nanofibers for TEM observation were prepared by direct electrospinning onto a copper grid.

2.3.4. Differential scanning calorimetry (DSC)

The thermal properties of dried PCEC/Fe_3O_4 membranes with different Fe_3O_4 contents were measured on a differential scanning calorimeter (NETSCZ 204, NETSCZ, Germany). All measurements were performed under nitrogen atmosphere.

2.3.5. Scanning electron microscopy (SEM) observation

The morphology of the electrospun PCEC/Fe_3O_4 nanofibers was observed on an electron scanning microscopy (SEM, JEM-100CX, Japan) at 20 kV. Before observations, the surface of each sample was coated with a thin layer of gold.

2.3.6. In vitro degradation

For the analysis of hydrolytic degradation, about 100 mg of the composite membranes with different Fe_3O_4 loadings were weighed (W_0) and then placed in a glass bottle containing 50 mL of degradation media (PBS, 0.01 M, pH = 7.4). The tests were performed by placing this on a rotary shaker with a shaking rate of 100 r/min at 37 °C. The degradation media were changed every week. At each