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Icariin immobilized electrospinning poly(L-lactide) fibrous membranes via polydopamine adhesive coating with enhanced cytocompatibility and osteogenic activity

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ABSTRACT

In this study, icariin (ICA), one of the main active ingredients of Herba Epimedii for osteogenesis, was applied to functionalize electrospinning poly(L-lactide) (PLLA) fibrous membrane via an intermediate layer of polydopamine (PDA) to obtain enhanced cytocompatibility and osteogenic activity. For this purpose, an array of PDA-coated PLLA fibrous membranes (PLLA-0.5PDA, PLLA-1PDA, PLLA-2PDA, PLLA-5PDA) and ICA-modified PLLA-2PDA fibrous membranes (PLLA-2PDA-10ICA, PLLA-2PDA-20ICA, PLLA-2PDA-40ICA) were successively prepared. Successful modification of PDA and ICA onto PLLA fibrous membranes was verified by field emission scanning electron microscope (FESEM), thermogravimetric analysis (TGA) and X-ray photoelectron spectroscopy (XPS). Besides, the hydrophilicity as well as tensile properties of PLLA fibrous membrane were improved after surface modified with PDA and ICA. In vitro cells culture experiments revealed that the adhesion, proliferation and osteogenic differentiation of MC3T3-E1 cells on the PLLA fibrous membrane were significantly improved by successively immobilized with PDA and ICA. Moreover, the concentration of ICA immobilized on the fibrous membranes has the complicated effects on the MC3T3-E1 cells behavior. The PLLA-2PDA-ICA fibrous membranes with low ICA concentration promoted the cell adhesion and proliferation, but on the contrary, those with high ICA concentration were more beneficial to the enhancement in ALP activity and calcium deposition.

1. Introduction

Bone defect or trauma is a serious public health issue nowadays, and the clinical demands for bone regenerative treatments have increased annually due to an aging population and traffic accidents involving bone damage [1–2]. Therefore, the reconstruction of bone defects still remains a main challenging in clinical treatment. In recent years, with the proposal and development of bone tissue engineering, significant attention has been devoted to developing artificial materials to treat bone defects. In general, the basic characteristics of a bone tissue engineering scaffold should possess a porous three-dimensional (3D) structure, favorable mechanical properties, biodegradability as well as good cytocompatibility. Moreover, it's also crucial to design functionalized scaffolds with excellent capacity of osteogenic activity [3].

Poly(L-lactide) (PLLA), approved by U.S. Food and Drug Administration (FDA) for clinical use, has been widely used as the bone tissue engineering scaffold due to its biodegradability, biocompatibility, attractive mechanical properties and processibility [4–7]. However, some problems still remain, such as the hydrophobicity, inferior cell affinities and lacking of osteogenic activity of PLLA, which are far from meeting the harsh demands of bone tissue engineering scaffold in clinical treatment [8–9]. In this regard, it is necessary to modify PLLA via directly or indirectly incorporating bioactive agents to obtain functional scaffold with the capability of meeting the strict requirements of bone regeneration.

In recent years, the mussel-inspired chemistry has been reported as a versatile modification approach, which uses dopamine as a powerful surface modifier and is irrespective of material type [10–11]. Dopamine, containing catechol and amine functional groups, can form a tightly adherent polydopamine (PDA) coating onto a substrate by self-polymerization in alkaline solution [12]. More importantly, the PDA adhesive coating can act as an assistant platform for secondary reaction and surface functionalization under mild conditions [13–15]. So far there are reports that utilizing the reactivity of PDA layer to immobilize various types of bioactive factors (growth factors or biomolecules) onto substrates to endow materials with superior performance. For examples, Lee et al. grafted recombinant human bone morphogenetic protein (rhBMP2) onto a 3D printed polycaprolactone scaffold based on a PDA layer to enhance both the proliferation and osteogenic activity of

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mouse embryo osteoblast precursor (MC3T3-E1) cells [16]. Poh et al. demonstrated that vascular endothelial growth factor (VEGF) was immobilized onto titanium alloy substrate via PDA coating, which was beneficial in promoting vasculature formation and new bone tissue formation [17]. However, there are also some limitations with the clinical use of growth factors, such as the expensive cost and short-lived bioactivity [18]. Thus, it is desirable to utilize some inexpensive and natural available medicaments to enhance the bioactivity of biomaterials. As we reported that the surface functionalization of PDLLA membrane with deferoxamine (DFO), a metal chelating agent for osteogenesis and angiogenesis, via the PDA coating can enhance the proliferation of both MC3T3-E1 cells and human umbilical vein endothelial cells (HUVECs) [19].

Icariin (ICA), one of the main active ingredients of Herba Epimedii, is extensively researched in the field of bone healing and applied in many Chinese formulas for treatment of osteoporosis [18,20–21]. Previous reports have shown that ICA has therapeutic effect on stimulating the proliferation of osteoblast and inducing osteogenic differentiation [22–24]. Besides, ICA possibly exerts its osteogenic effects through the induction of BMP-2 gene expression [25]. Along with the further researches, more and more studies begin to pay close attention to the modification of biomaterials with ICA to endow the materials with positive effects such as osteogenic activity. As Wu et al. developed a new bone repair scaffold through mixing of ICA and chitosan/hydroxyapatite (ICA-CS/HA) using a freeze-drying technique. The scaffold showed favorable osteogenic activity in vivo and hence led to new bone formation [26]. Besides, Zhang et al. prepared ICA loaded porous β-TCP ceramic (ICA/β-TCP) scaffold by directly soaking method. The results of intramuscular implantation in rats revealed that no obvious osteogenic evidence was detected in β-TCP scaffolds, but new bone formation was observed in ICA/β-TCP scaffolds [18].

Electrospinning is a facile technique that can produce 3D fibrous membranes composed by micro- or nano-fibers with high surface areas and interconnected pores [27–35]. For the guidance of bone regeneration, this technique has been widely used to obtain fibrous membranes with controllable fiber morphology, adjustable fiber orientation and devisable fiber topological structure by changing processing parameters. What's more, electrospinning fibrous membranes can closely mimic the structure of native extracellular matrix to promote cell adhesion and proliferation.

To date, various methods, including freeze-drying [26], directly soaking [18], solvent casting [22] and etc. have been applied to load ICA into biomaterials. However, the utilization of PDA adhesive coating to immobilize ICA onto biomaterial surface has seldom been reported. In this current work, ICA was designed to be immobilized onto the surface of PLLA fibrous membrane fabricated by electrospinning technique based on the intermediate layer of PDA. The morphology, thermal stability, surface composition, porosity, hydrophilicity and tensile properties of the unmodified and modified PLLA fibrous membranes were evaluated in details. The cytocompatibility and osteogenic activity of the as-prepared fibrous membranes was comprehensively studied in terms of the adhesion, proliferation and viability, as well as ALP activity and calcium deposition of the MC3T3-E1 cells. The results would supply a research basis for the further use of the ICA functionalized PLLA fibrous membrane as the scaffold with promoted osteogenesis for bone defects.

2. Materials and methods

2.1. Materials and reagents

PLLA (M_w = 150,000) was purchased from Jinan Daigang Biomaterial Engineering Co., Ltd. Tris (hydroxymethyl) aminomethane (Tris) and dopamine were received from Sigma-Aldrich and used as received. ICA ($C_{33}H_{40}O_{15}$) was obtained from Beijing century Aoke Biological Technology Co., Ltd. All other reagents obtained from Guangzhou Chemical Reagent Plant are analytical grade.

2.2. Preparation of the unmodified and modified PLLA fibrous membranes

PLLA fibrous membrane was fabricated via electrospinning technique using *N*,*N*-dimethylformamide and dichloromethane (DMF/DCM = 1/3 by volume) as a co-solvent. A 7 wt% PLLA solution was added into a syringe fitted with 22 gauge needle, and the flow rate was controlled at 1 mL/h by a syringe pump. A positive voltage of 18 kV was employed to the needle and a grounded stainless steel plate wrapped with aluminum foil was used as a collector that is 12 ± 2 cm in distance apart from the tip of the syringe. The obtained PLLA fibrous membrane was dried in vacuum for 24 h at 40 °C.

The PLLA fibrous membranes (7 cm × 7 cm) were then separately submerged in 0.5, 1, 2 and 5 mg/mL dopamine solution (dissolved in 10 mM Tris–HCl buffer, pH = 8.5) with mild shaking for 24 h to obtain PDA modified PLLA fibrous membranes, which were labelled as PLLA-0.5PDA, PLLA-1PDA, PLLA-2PDA and PLLA-5PDA, respectively. The reaction mechanism of the oxidative self-polymerization of dopamine was demonstrated in Fig. 1. The loosely bound PDA was vigorously washed with distilled water several times. The PLLA-PDA fibrous membranes were obtained after being dried in vacuum at 40 °C for 24 h.

ICA solutions with concentration of 10, 20 and 40 μg/mL were obtained by dissolving ICA in ethanol/Tris-HCl buffer (4/6 by volume). The above obtained PLLA-2PDA membrane was chosen and further immersed into the ICA solutions with shaking for 24 h to obtain PLLA-2PDA-ICA fibrous membranes. Then, the fibrous membranes were taken out and rinsed with deionized water. The final ICA modified fibrous membranes were labelled as PLLA-2PDA-10ICA, PLLA-2PDA-20ICA and PLLA-2PDA-40ICA, which were dried in vacuum at 40 °C for 24 h.

As comprehensive described above, an outline of the preparation route of the modified PLLA fibrous membranes and relatively reaction mechanism of PDA oxidative self-polymerization was illustrated in Fig. 1.

2.3. Characterization of the unmodified and modified PLLA fibrous membranes

Surface morphologies of the as-prepared fibrous membranes were observed by field emission scanning electron microscope (FESEM, ULTRA 55, Carl Zeiss, Germany). Thermal stability was tested by a thermogravimetric analyzer (TGA, TG209F1, Netzsch, Germany) between 22 °C and 800 °C at a heating rate of 10 °C/min under nitrogen atmosphere. X-ray photoelectron spectrum (XPS, Thermo ESCALAB-250 System, Australia) was conducted using a Kα source (1486.6 eV), and the final XPS data were analyzed by XPS PEAK41 based on the Gaussian-Lorentzian composite function. The porosity of the membranes was performed according to the previous literature [36]. Tensile properties were measured on a universal mechanical testing machine (AG-1, SHIMADZU, Japan) with a crosshead speed of 3 mm/min. The surface hydrophilicity of the fibrous membranes was determined using a goniometer (DSA100, Kruss, Germany) by determining surface contact angle.

2.4. In vitro MC3T3-E1 cell culture

MC3T3-E1 cells were cultured in alpha-modified Eagle's medium, without ascorbic acid, with 10% (v/v) fetal bovine serum and 1% (v/v) pen-strep solution at 37 °C with 5% CO_2. To induce cell differentiation, the cells were cultured with osteogenic medium and then were incubated for long terms (21 days). Prior to cell experiments, the samples placed into a 24-well plate were sterilized by ultra-violet light for 1 h and subsequently immersed in cell culture medium for 2 h. Cells were seeded on the fibrous membranes at the density of 1×10^4 cells/well and cultured for short term (1, 4 and 7 days) and long term (21 days). The cell culture medium was changed every two days.