Biomaterials 32 (2011) 3404–3412

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Cell affinity for bFGF immobilized heparin-containing poly(lactide-co-glycolide) scaffolds

Hong Shen, Xixue Hu, Fei Yang, Jianzhong Bei, Shenguo Wang*

BNLMS, State Key Laboratory of Polymer Physics & Chemistry, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China

ARTICLE INFO

Article history:
Received 29 December 2010
Accepted 13 January 2011
Available online 5 February 2011

Keywords:
bFGF
Immobilization
Slow release
Heparin
PLGA
Scaffold

ABSTRACT

In order to effectively and uniformly immobilize basic fibroblast growth factor (bFGF) to thick PLGA scaffold, the heparin-conjugated PLGA (H-PLGA) was synthesized at the first by reaction between heparin and a low molecular weight PLGA. Then heparin-containing PLGA (H-PLGA/PLGA) scaffold was fabricated by blending the H-PLGA with a high molecular weight PLGA. Finally, bFGF was immobilized on the H-PLGA/PLGA scaffold mainly by static electricity action between them. The effect of H-PLGA content on bFGF binding efficiency of the H-PLGA/PLGA scaffolds was investigated. It was found that bFGF binding efficiency increased with increasing H-PLGA content. The bound bFGF can release in vitro slowly from the H-PLGA/PLGA scaffolds and last over two weeks. The released bFGF has still preserved its bioactivity. The attachment and growth of mouse 3T3 fibroblasts on the H-PLGA/PLGA scaffolds were better than that on the PLGA scaffold, however bFGF immobilized H-PLGA/PLGA scaffolds showed much better cell affinity. Therefore, the method to use the H-PLGA/PLGA scaffold for immobilizing bFGF is not only effective for slow delivering bFGF with bioactivity, but also can be used for fabricating thick scaffold where bFGF could be combined and uniformly distributed.

1. Introduction

Recently, the research of combination of growth factors into polylactone-type biodegradable polymeric scaffolds had been extensively noticed by biomaterial and tissue engineering researchers [1–4]. Polylactone-type biodegradable polymers, such as poly(L-lactide) (PLLA), polyglycolide (PGA) and their copolymer poly(lactide-co-glycolide) (PLGA) etc possess non-toxicity, low immunogenicity, good mechanical property, and adjustable degradation rate [5,6], however, lack of cell recognition sites, poor hydrophilicity and lower surface energy of the polymers will affect cell to attach and grow on the polymeric scaffolds [7,8]. On the other hand, it is considered that growth factors are polypeptides that can transmit signals to modulate cellular activities [9]. Administration of exogenous growth factors also showed potential therapeutic results for tissue regeneration, bone healing, wound healing, as well as angiogenesis in vivo [10–14], and had positive effect on adhesion, proliferation, differentiation, migration, and gene expression of a variety of cell types in vitro [13,15–18]. However, since growth factor is easy to denature in presence of water and under higher temperature, bioactivity of the growth factor cannot keep for a long term when it is injected into the body by aqueous solution state. In result, it could be only retained at the wound sites for very short duration and half-life of the growth factor became very short caused by its susceptibility to enzymatic and thermal degradation in vivo [19–21]. Therefore, an effective growth factor delivery system is required to overcome shortcoming of the growth factor for clinical therapy.

Considering most of polymers are hydrophobic, a hopeful strategy is to combine growth factor into a hydrophobic polymer for avoiding the growth factor to contact water by the hydrophobic polymeric barrier. After combination of growth factor into polylactone-type polymeric scaffolds, the scaffolds can possess not only adequate mechanical strength, required biodegradation rate and morphological structure, but also effective delivery behavior of the growth factor for actively guiding and accelerating cell attachment, migration, proliferation and differentiation in the scaffolds. However, the challenge of the technique is how to incorporate the water-soluble growth factors into the hydrophobic polymeric scaffolds evenly.

It was reported that growth factors can be incorporated directly into the polymer scaffolds during [2,22–24] or after the scaffold fabrication [25–27]. However, because fabrication of polymer scaffolds must use organic solvent, bioactivity of the growth factor will be damaged and reduced. On the other hand, the poor hydrophilicity and lack of functional group of the polymers often result in lower growth factor loading efficiency and the growth

* Corresponding author. Tel./fax: +86 10 62581241.
E-mail address: wangsg@iccas.ac.cn (S. Wang).

0142-9612/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2011.01.037

factor could not be tightly bound to the polymers by solution dipping method after the scaffold fabrication.

Previously authors ever reported a plasma treatment combining with growth factor anchorage method, which can effectively immobilize growth factor on PLGA scaffolds [28,29]. This method is rapid, clean and without organic solvent pollution. However, using the plasma treatment method the depth of immobilized growth factor in the scaffold is limited because of straight irradiation and weaker trans-permeability of the plasma ray. In result the inner pore surface of the thicker scaffold will be difficult to be modified, on the other hand, the outer surface of the scaffold will be possible to deform and degrade seriously if high power and long treatment time are administrated [30]. Therefore, although the plasma treatment combining with growth factor anchorage method is an effective method for immobilizing growth factors on the PLGA scaffolds, the defect of the method is the treated thickness of the scaffolds is limited. So, the urgent requirement is to develop a more effective method for uniformly immobilizing growth factor into thick scaffolds.

It was reported that introducing heparin into polylactone-type scaffolds can be easy to immobilize growth factor into the scaffolds by ability of heparin binding growth factor [31,32]. Heparin is a highly sulfated macromolecular polysaccharide which can associate with the cell surface and it is one component of extracellular matrix [31,33]. It is well accepted that the specific electrostatic interactions can occur between the negatively charged sulfate groups of heparin and positively charged amino acid residues of proteins [31,33,34], and the electrostatic interaction can enhance binding affinity of the heparin to a number of growth factors such as bFGF, vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), nerve growth factor (NGF), bone morphogenetic proteins (BMPs) and enable the growth factors to diffuse out in a sustained manner [31,35,36].

Heparin can be introduced into the polylactone-type polymeric scaffolds by physical sorption, ion reaction and covalent binding, but the combined heparin by simple physical sorption method is unstable and uneven compared with other methods. Sometimes the stability of heparin bound on the polymeric scaffolds by ion reaction also could not meet the application demand. Although among three of them, the covalent bound heparin was the most stable, it is difficult to directly conjugate large number of heparin into the polymeric scaffolds by chemical method since there were very few functional groups (only two end groups) in backbone of the polymers, especially in the case of using high molecular weight polylactone-type polymers.

In this research a modified method for immobilizing growth factor was developed. At the first, a lower molecular weight PLGA was used to synthesize heparin-conjugated PLGA (H-PLGA). Then the H-PLGA was used to blend with a high molecular weight PLGA to obtain heparin-containing PLGA (H-PLGA/PLGA). By means of adjusting component ratio of the H-PLGA and the high molecular weight PLGA, a series of H-PLGA/PLGA scaffolds which containing different content of heparin were obtained. Finally, bFGF was bound into the H-PLGA/PLGA scaffolds to fabricate bFGF immobilized H-PLGA/PLGA scaffolds. The bFGF release profile of the bFGF immobilized H-PLGA/PLGA scaffolds as well as bioactivity of the released bFGF were determined, and then adhesion and growth of mouse 3T3 fibroblasts in vitro in the bFGF immobilized H-PLGA/PLGA scaffolds were determined, compared and discussed.

2. Materials and methods

2.1. Materials

L-Lactide and glycolide were purchased from PURAC (the Netherlands) and purified by recrystallization twice in dried ethyl acetate. High molecular weight PLGA (Mw = 127,000, molar ratio of lactyl/glycotyl = 70/30) was prepared by ring-opening polymerization of L-lactide and glycolide under high vacuum at 160 °C for 20 h in the presence of stannous octoate (SIGMA, German) as catalyst (0.05 wt% of L-lactide and glycolide) [37]. Lower molecular weight PLGA (Mw = 33,000, molar ratio of lactyl/glycotyl = 70/30) was prepared also by ring-opening polymerization of L-lactide and glycolide under 160 °C but for 30 min and concentration of stannous octoate was 0.1 wt% of L-lactide and glycolide. Heparin sodium salt (Beijing Biodee Biotechnology Co., Ltd, China, produced by porcine intestinal mucosa, 150 unit/mg), dicyclohexylcarbodiimide (DCC, Aldrich), 4-(dimethyl amino) pyridine (DMAP, Aldrich) and all other chemicals were used as received. The 1,4-dioxane, chloroform and other reagents were of analytical quality and directly used without further treatment.

2.2. Synthesis of H-PLGA

H-PLGA was prepared by direct coupling reaction of DCC/DMAP chemistry according to the reference [38]. Briefly, 0.6 g heparin was firstly dissolved in a mixture of formamide (30 mL) and *N,N*-dimethylformamide (30 mL), and then 0.6 g low molecular weight PLGA was dissolved in a mixture of formamide (100 mL) and *N,N*-dimethylformamide (100 mL). DCC (0.01 g) and DMAP (0.006 g) were added to above heparin solution and stirred for 10 min. Then, the above PLGA solution was dropped into the reaction solution system and stirred at 50 °C for 12 h under nitrogen atmosphere. After the coupling reaction, the reaction system was concentrated and then precipitated using excess ethanol. After the precipitate was washed by distilled water, the precipitate was dissolved again by chloroform and the produced solution was re-precipitated using excess ethanol. Finally, the precipitate was filtered out off the system and dried at 35 °C for 24 h in vacuum for eliminating the residual solvent to obtain the H-PLGA.

2.3. Preparation of H-PLGA/PLGA film and scaffold

H-PLGA/PLGA film was fabricated by a solution casting technique. Firstly, a blend of H-PLGA and high molecular weight PLGA with certain weight ratio was dissolved in chloroform to form 5 wt% composite solution. Then the blend solution was cast into a poly (tetrafluoroethylene) (PTFE) mould. After solvent had evaporated in air at room temperature, the formed film was removed from the mould and performed de-solvent thoroughly under vacuum at room temperature for 48 h.

H-PLGA/PLGA scaffold was manufactured by an improved solid–liquid phase separation method. Firstly H-PLGA and high molecular weight PLGA with certain weight ratio were dissolved in dioxane to form 8 wt% H-PLGA/PLGA blend solution. Then the blend solution was pushed into a column container which was full of a certain weight of sieved NaCl granules (200–280 μm) to form a composite and then the composite was maintained at 0 °C over 24 h to perform solid–liquid phase separation completely. After solvent was removed by freeze-drying for 3 days, the formed matrix column was put into distilled water to leach the NaCl out. The distilled water was renewed every 3 h until no chloric ion could be detected by dropping of $AgNO_3$ aqueous solution. The fabricated H-PLGA/PLGA scaffold was dried and kept in a desiccator for usage.

2.4. Determination of contact angle and porosity

Contact angle of various films to deionized water was measured on air surface of the films using a FACE CA-D-type Contact Angle Meter (Kyowa Kaimenka-gaku Co., Ltd). Ten independent determinations at different sites of a film were averaged.

Porosity of various PLGA scaffolds was determined according to a method reported previously [39]. At first, the scaffold was cut into short column with a certain diameter (D) and height (H), and the volume (V_w) of the scaffold including the pore volume (V_p) and the skeleton volume of the scaffold (V_s) were calculated. A density bottle was filled with ethanol (density ρ_e) at 30 °C and weighed (W_1). The scaffold sample (weight W_s) was put into the above density bottle and kept at 30 °C for 1 h under vacuum to remove any trapped air in the pores/tubules. Then the density bottle was filled with ethanol and kept at 30 °C again for 30 min and all the overflowed ethanol was cleaned away carefully. Finally the density bottle was weighed again (W_2). Parameters of the scaffold including the volume of the whole scaffold (V_w), the volume of pores (V_p), the volume of the scaffold skeleton (V_s), and the porosity (ε) of the scaffold were calculated as follows:

$$V_w = V_p + V_s = \pi \times (D/2)^2 \times H$$

$$V_s = (W_1 + W_s - W_2)/\rho_e$$

$$\varepsilon = 1 - V_s/V_w$$

2.5. Characteristics of heparin on the surface of H-PLGA/PLGA scaffold

Heparin content on the surface of H-PLGA/PLGA scaffolds was determined by the toluidine blue colorimetric method [38,40]. The column type H-PLGA/PLGA scaffold with 8 mm of diameter and 4 mm of height was placed in 1 mL of 0.2% NaCl solution, then 1 mL of toluidine blue solution (0.05 g of toluidine blue was dissolved in 1000 mL