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The bioactivity of rhBMP-2 immobilized 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 100080, China

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ABSTRACT

In this study, immobilization of rhBMP-2 on polylactone-type polymer scaffolds via plasma treatment was investigated. To introduce proper functional groups on the surface of poly(lactide-co-glycolide) (PLGA) matrix, PLGA films were treated under different atmospheres, such as oxygen, ammonia and carbon dioxide, respectively, and then incubated in rhBMP-2 solution of de-ionized water. The effect of various plasma-treated PLGA films on binding rhBMP-2 was investigated and compared. It was found that PLGA binding ability to rhBMP-2 was enhanced by carbon dioxide and oxygen plasma treatment, and the binding ability of the oxygen plasma-treated PLGA (OT-PLGA) to rhBMP-2 was the strongest after oxygen plasma treating for 10 min under a power of 50 W. The changes of surface chemistry and surface topography of PLGA matrix induced by oxygen plasma treatment played main roles in improving the PLGA binding ability to rhBMP-2. The stability of rhBMP-2 bound on OT-PLGA film was determined under a dynamic condition by a Parallel Plate Flow Chamber. The result showed that the rhBMP-2 had been immobilized on the OT-PLGA film. Mouse OCT-1 osteoblast-like cell as a model cell was cultured on the rhBMP-2 bound OT-PLGA (OT-PLGA/BMP) in vitro, which showed that the bound rhBMP-2 via oxygen plasma treatment was bioactive. Depending on hydrophilicity and rich polar O-containing groups of the OT-PLGA scaffold, different amount of rhBMP-2 could be evenly immobilized on the surface of the OT-PLGA scaffold. The immobilized rhBMP-2 had stimulated differentiation of OCT-1 cell and accelerated process of mineralization of OCT-1 cell in the scaffold. It revealed the rhBMP-2 immobilized PLGA scaffold had good cell affinity.

1. Introduction

Tissue engineering aims at the repair, restoration or regeneration of damaged tissue function using cells, scaffolds and growth factors alone or in combination [1,2]. Many results of experimental and clinical researches show that the success of any tissue engineering approach mainly relies on the delicate and dynamic interplay among these three components [3–5]. Therefore, one of the significant challenges for tissue engineering is to design and fabricate suitable biodegradable scaffolds which not only possess adequate biodegradation rate, mechanical strength and morphological structural but also can effectively deliver specific growth factor to actively guide and control cell attachment, migration, proliferation and differentiation. Polylactone-type biodegradable polymers, such as poly(L-lactide) (PLLA), polyglycolide (PGA) and their copolymer poly(lactide-co-glycolide) (PLGA), are extensively studied as scaffold materials for tissue engineering [6–9], since they possess good mechanical property, low immunogenicity, non-toxicity and adjustable degradation rate. However, cytocompatibility of the polylactone-type biodegradable polymer scaffold is not good due to the lack of cell recognition sites on their surface. So it is far from the ideal scaffold which can bind growth factor on it and make the scaffold supply biological signals for guiding and accelerating cell attachment, migration, differentiation and proliferation.

Bone morphogenetic protein (BMP) is a well used growth factor that plays a crucial role in bone formation and repair [10,11]. BMP regulate cell growth and differentiation of a variety of cell types including osteoblasts and chondrocytes [10,12]. In particular, BMP-2, as member of the BMP family, has become one of the most potent members of the BMP family due to the induction of bone formation in vivo by promoting the maturation of committed cells to become more differentiated osteoblasts [10,12,13]. Moreover, since recombinant human bone morphogenetic protein-2 (rhBMP-2) is available in large quantities and lacks risks associated with matrix extracts related to potential viral infection, BMP-2 has been extensively studied in tissue engineering application [14–17]. Recently, to improve polylactone-type biodegradable polymer scaffolds, a number of strategies including physisorption, ionic interaction, and blending have been designed to immobilize BMP-2 on the polylactone-type scaffolds [13,18–21]. The challenge is how

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

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to tightly incorporate the water-soluble protein into the hydrophobic polymer scaffolds evenly. BMP-2 can be incorporated directly into the polymer scaffolds at [20,22,23] or after fabrication [21,24,25]. However, use of organic solvent during the polymer scaffolds fabrication process will damage bioactivity of the BMP-2. On the other hand, the poor hydrophilicity and lack of functional group of the polymers often results in low loading efficiency and un-tight binding of BMP-2 by solution dipping method after fabrication. Aimed to improve binding ability of the polylactone-type polymer scaffold to the BMP-2, surface modification of the polylactone-type polymers has been developed [10]. However, since the functional groups are absent in the backbone of the polymers, it is difficult to modify surface property of polylactone-type polymers by common chemical method.

The present authors previously have reported an effective method to immobilize basic fibroblast growth factor (bFGF) on PLGA scaffold [26]. In the method, the PLGA scaffold was pretreated by carbon dioxide (CO_2) plasma and then was anchored with bFGF. bFGF had been immobilized on the PLGA scaffold by electrostatic interaction between the basic group of bFGF and the rich acidic carboxylic group of the PLGA scaffold which resulted from CO_2 plasma treatment of the polymer scaffold. The immobilization method of CO_2 plasma treatment combining with bFGF anchorage is a rapid, clean and non-solvent pollution method. Plasma treatment can be used to introduce some specific element or functional group onto surface of a polymer only by selecting and applying some suitable gas [27–29]. Surface property of a material such as wettability, topography, surface charge states and biocompatibility can be effectively modified and adjusted by controlling parameters of plasma treatment, although bulk properties of the material will be little changed. Since some specific functional groups such as amine, carbonyl, carboxyl, hydroxyl, as well as ether can provide special chemical reactivity and varying physical properties of the surface, it is benefit for functionalization of the surface with bioactive molecules. It is considered that the plasma treatment combining with anchorage of bFGF technique is hopeful to extend to immobilize other growth factors onto the PLGA scaffold by only choosing and applying other suitable gases for the plasma treatment.

In this study, firstly the effect of various plasma treatments on anchoring rhBMP-2 on PLGA was investigated and compared. Based on the result that oxygen plasma treatment could provide proper property for surface of PLGA matrix for anchoring rhBMP-2, the oxygen plasma treatment was chosen to pretreat PLGA for immobilizing rhBMP-2. Then the binding rhBMP-2 ability of the oxygen plasma-treated PLGA matrix was investigated in vitro by the gradient-binding experiment. Moreover, the stability of rhBMP-2 bound to PLGA film was further tested under shear stress by a Parallel Plate Flow Chamber [30,31], and bioactivity of the immobilized rhBMP-2 was evaluated by measuring ALP activity of mouse OCT-1 osteoblast-like cell cultured on the immobilized rhBMP-2-PLGA film. Finally, rhBMP-2 immobilized PLGA scaffold was prepared and the influence of immobilized rhBMP-2 on the growth of mouse OCT-1 cell was investigated.

2. Materials and methods

2.1. Materials

PLGA (molar ratio of lactyl/glycotyl = 70/30, Mw = 120,000) was prepared by ring-opening polymerization of L-lactide (PURAC, Netherlands) and glycolide (PURAC, Netherlands) under high vacuum at 160 °C for 20 h in the presence of stannous octoate (SIGMA, German) as catalyst (0.05 wt%) [32].

2.2. Preparation of PLGA film and scaffold

The PLGA film with dense structure was prepared by casting 5 wt% PLGA chloroform solution into a poly (tetrafluoroethylene) (PTFE) mould. After solvent evaporation in air at room temperature the formed film was removed from the mould and performed removing residual solvent thoroughly under vacuum at room temperature for 48 h. Thickness of the obtained PLGA film was 0.1 mm.

The PLGA scaffold was manufactured by an improved solid–liquid phase separation method [33]. A certain weight of sieved NaCl granules (diameter 200–280 μm) was added into 5% (w/v) solution of the PLGA in dioxane, then the slurry was maintained at 0 °C for over 24 h to perform solid–liquid phase separation completely. After the solvent was removed by freeze–drying for 3 days, the formed matrix 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, and then a porous structured PLGA scaffold was fabricated. At the same time, due to the miscibility between the dioxane and water, the residual dioxane was extracted out and replaced with water. Finally, the fabricated porous structured PLGA scaffold was dried and kept in a desiccator for usage.

2.3. Plasma treatment

Plasma treatment of the PLGA film and scaffold was carried out on Samco Plasma Deposition (Model PD-2, 13.56 MHz) under different atmosphere, such as oxygen, ammonia and carbon dioxide, respectively. PLGA film or scaffold was placed on the electrode in the plasma chamber. The chamber was evacuated to less than 10 Pa before filling with gas. After gaseous pressure of the chamber was stabilized to 20 Pa, a glow discharge plasma was created by controlling the electric power at a radio frequency of 13.56 MHz for a predetermined time. Finally, the plasma-treated sample was further exposed to the atmosphere for another 10 min before the sample was taken out from the chamber.

2.4. Determination of bound rhBMP-2

Both plasma-treated and untreated PLGA films were cut into disks with a diameter of 7 mm and placed in the bottom of a 96-well plate. 100 μl of rhBMP-2 (PeproTech, USA) solutions with increasing concentration from 0 to 120 μg/ml were used for incubation. After the PLGA samples were incubated in the rhBMP-2 solution for 1 h at room temperature on a shaker, the PLGA samples were rinsed for three times with phosphate buffer saline (PBS, pH 7.4) and then the binding proteins were measured by a modified ELISA assay method [34,35].

Briefly, after 200 μl of 5% BSA/PBS was respectively added in the PLGA sample contained wells of the 96-well plate and incubated for 2 h on a platform shaker, the wells were washed for three times with PBS. Then another 200 μl (1:1000 dilution) anti-polyHistidine antibody (Sigma, USA) was added in the wells at room temperature for additional 2 h incubation. After unbound primary antibody was removed by three washes with PBS, the secondary antibody of 200 μl of ALP conjugated goat-anti-mouse IgG (1:10000 dilution, Sigma, USA) was added to each well. The ALP reaction product was developed by incubation with para-nitrophenylphosphate (pNPP) (Sigma, USA) at room temperature for 10 min and then the absorbance of the reaction product at 405 nm was determined by using plate reader (TECAN, SUNRISE, Austria).

$$\Delta OD405 = OD405s - OD405c$$

where OD405s was the absorbance derived from the experimental sample which incubated in the solutions with concentration of rhBMP-2 > 0 μg/ml, and OD405c was the absorbance derived from the control which incubated in the solution with concentration of rhBMP-2 = 0 μg/ml, respectively.

In the present research, the untreated PLGA and oxygen, ammonia as well as carbon dioxide plasma-treated PLGA were respectively abbreviated as UT-PLGA, OT-PLGA, AT-PLGA and CT-PLGA. The rhBMP-2 bound PLGA prepared under without plasma pretreatment and with oxygen plasma pretreatment were abbreviated as UT-PLGA/BMP and OT-PLGA/BMP, respectively.

2.5. Stability determination of bound rhBMP-2

According to previous reports [30,31], stability of bound rhBMP-2 was determined under shear stress by a circuit flow system in a Parallel Plate Flow Chamber (PPFC). Firstly, the UT-PLGA/BMP and OT-PLGA/BMP films with a diameter of 10 mm were immobilized on bottom glass plate of PPFC and the top glass plate was assembled in the PPFC. A certain distance between both the parallel glass plates was kept. After all bubbles in the circuit flow system were removed carefully, PBS solution (pH 7.4) was circularly flowed in the system initiated by a peristaltic pump. The flowing rate of the PBS solution was controlled by adjusting the peristaltic pump to keep 11.5 N/m^2 of shear stress in the PPFC. At the same time, temperature of the PPFC was also controlled by adjusting heating voltage to keep 37 °C. After the films had been exposed to the shear stress for a certain time, they were taken out from the glass plate and the retained rhBMP-2 was assayed. Finally, the stability of the bound rhBMP-2 on UT-PLGA or OT-PLGA film was compared.